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(54) Title: HEXADECASACCHARIDE–PROTEIN CONJUGATE VACCINE FOR *SHIGELLA DYSENTERIAE* TYPE 1

(57) Abstract

A

The present invention pertains to isolated natural, modified natural or synthetic oligo– or polysaccharides which have [3)–α–L–Rha*p*–(1→2)–α–D–Gal*p*–(1→3)–α–D–Glc*p*NAc–(1→3)–α–L–Rha*p*–(1→]$_n$ subunits and which are structurally related to and/or antigenically similar to an antigenic determinant of the O–specific polysaccharide of *Shigella dysenteriae* type 1. The oligo– or polysaccharides may be conjugated to a carrier to form conjugates. These oligo– or polysaccharides and conjugates thereof are immunogenic and elicit serum antibodies that are bactericidal or bacteriostatic against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, and are useful in the prevention and treatment of shigellosis. These oligo– or polysaccharides and conjugates thereof, and the antibodies which they elicit, are also useful for diagnosing shigellosis caused by *Shigella dysentariae* type 1.

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Hexadecasaccharide-Protein Conjugate Vaccine for *Shigella dysenteriae* type 1

FIELD OF THE INVENTION

This invention relates to compositions and methods for eliciting an immunogenic response in mammals, including responses which provide protection against, or reduce the severity of, bacterial infections. More particularly it relates to the use of oligo- or polysaccharides, in particular oligo- or polysaccharides containing the repeating unit [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→], obtained from natural sources and/or through synthesis, and conjugates thereof, to induce serum antibodies having bactericidal (killing) activity against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1. These saccharides and/or conjugates thereof are useful as vaccines to induce serum antibodies which have bactericidal or bacteriostatic activity against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, and are useful to prevent and/or treat shigellosis caused by *Shigella dysenteriae*.

The present invention also relates to diagnostic tests for shigellosis using one or more of the oligo- or polysaccharides, conjugates or antibodies described above.

BACKGROUND OF THE INVENTION

The most successful of all carbohydrate pharmaceuticals so far have been the carbohydrate based, antibacterial vaccines.[1] The basis of using carbohydrates as vaccine components is that the capsular polysaccharides and the O-specific polysaccharides on the surface of pathogenic bacteria are both protective antigens and essential virulence factors. The first saccharide-based vaccines contained capsular polysaccharides of *Pneumococci*: in the United States a 14-valent vaccine was licensed in 1978 followed by a 23-valent vaccine in 1983. Other capsular polysaccharides licensed for human use include a tetravalent meningococcal vaccine and the Vi polysaccharide of *Salmonella typhi* for typhoid fever. The inability of most polysaccharides to elicit protective levels of anti-carbohydrate antibodies in infants and adults with weakened immune systems could be overcome

by their covalent attachment to proteins that conferred T-cell dependent properties.[2] This principle led to the construction of vaccines against *Haemophilus influenzae* b (Hib) [3] and in countries where these vaccines are routinely used, meningitis and other diseases caused by Hib have been virtually eliminated [4]. Extension of the conjugate technology to the O-specific polysaccharides of Gram-negative bacteria provided a new generation of glycoconjugate vaccines that are undergoing various phases of clinical trials [5].

Shigella dysenteriae type 1 [6] is a human pathogen that is a major causative organism of endemic and epidemic dysentery worldwide. The severity of infections with shigellae may vary from mild watery diarrhea to dysentery with blood and mucus in the stool and severe cramps. Diarrhea is defined as four or more watery bowel movements daily. Dysentery is defined as fever, cramps, and the presence of blood, alone or with mucus, in the stools. Patients with shigellosis may have dysentery, diarrhea, or both simultaneously. Dysentery is associated with a higher mortality rate and has a greater effect on long-term growth retardation than does diarrhea [7].

The incidence of dysentery is higher in infants and young children with shigellosis than it is in adults. Watery diarrhea, the major symptom of shigellosis, is more frequent in adults. These findings suggest there is an age related increase in resistance to the more severe form of intestinal inflammation caused by the pathogen. Antibodies to lipopolysaccharides (LPS) are detected both in a higher percentage and at higher titers in individuals as they reach adulthood. These antibodies are probably stimulated by cross-reacting polysaccharides and by homologous organisms in areas in which disease caused by *Shigella* is rarely encountered [7].

Shigellosis is described as an inflammatory disease confined to the large intestine - systemic manifestations are thought to be medicated by release of lymphokines or bacterial products, such as LPS or Shiga toxin, into the circulation [7,8]. However, shigellosis, especially in infants, may not be confined to the intestine but may be caused by spread of the pathogen through the interstitial spaces of the submucosa into the blood [7]. *Shigella* organisms or their LPS have also been

found in the bloodstream or at extraintestinal sites such as blood, lymph nodes and spleen [8].

Although there is no consensus on the host factors that confer immunity to diseases caused by *Shigella*, the O-specific polysaccharide portion of the lipopolysaccharides of *Shigella* emerged as a possible protective antigen [9,10]. These polysaccharides were shown to be essential for the virulence of *Shigella* and it is now well-established that the protection is serotype specific. Since each serotype is characterized by a distinct O-specific polysaccharide, it is fair to say that protection is O-specific polysaccharide specific. Robbins et al. hypothesized that serum antibodies to the O-SP may confer protective immunity [7,8]. Indeed, the safety and immunogenicity of a protein conjugate of the O-specific polysaccharides of *S. sonnei, S. flexneri 2a,* and *S. dysenteriae* type 1 was demonstrated in human volunteers, and preliminary clinical trials have established the efficacy of these vaccines [11,12].

Studies in this laboratory have shown that protein conjugates of the O-specific polysaccharide of *Shigella dysenteriae*, which contains the repeating unit [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$, are capable of inducing serum antibodies that can offer host protection [8, 13].

Chemical synthesis may provide fragments of natural polymers that have the necessary geometry to mimic conformational determinants of the native polymer which may be useful in diagnostics or as components of vaccines. The synthesis of di- to penta-, tetra-, hexa-, octa-, and dodeca- saccharide fragments of the O-specific polysaccharide of *Shigella dysenteriae* type 1 [14,15,16] and the use of such synthetic oligosaccharides to map the carbohydrate binding specificity of anti O-specific polysaccharide specific murine monoclonal antibodies [17] have been previously reported. More recently, the synthesis of a hexadecasaccharide of *Shigella dysenteriae* type 1, consisting of consecutive tetrasaccharide repeating units, has been reported [18].

The immunogenicity of saccharides alone or as protein conjugates, is related to several variables: 1) species and the age of the recipient ; 2) molecular weight of the saccharide ; 3) density of the saccharide on the protein ; 4) configuration of the conjugate (single vs. multiple point attachment) ; 5) the

immunologic properties of the protein of the saccharide.

Because high molecular weight polysaccharides can induce the synthesis of antibodies from B-cells alone, they are described as T-independent antigens. Three properties of polysaccharides have been related to T-independence; 1) their repetitive polymeric nature which results in one molecule having multiple identical epitopes; 2) a minimal molecular weight that is related to their ability to adhere to and cross-link membrane-bound IgM receptors resulting in signal transduction and antibody synthesis; 3) resistance to degradation by mammalian enzymes. Most capsular polysaccharides are of comparatively high molecular weight (≥150 kD) and elicit antibodies in older children and in adults but not in infants and young children. O-SPs are of lower molecular weight (≤20 kD) and may be considered as haptens because they combine with antibody (antigenic) but do not elicit antibody synthesis (not immunogenic). The immunogenicity of O-SPs as conjugates may be explained by two factors: 1) the increase in molecular weight that allows the O-SP to adhere to a greater number of membrane-bound IgM and induce signal transduction to the B-cell; and 2) their protein component that is catabolized by the O-SP stimulated B cell resulting in a peptide-histocompatibility II antigen signal to T cells.

Synthesis of conjugates for use as vaccines in humans has special considerations. LPS is not suitable for parenteral administration to humans because of toxicity mediated by the lipid A domain. Initially, the O-SP was prepared by treatment of LPS with either acid hydrolysis or hydrazinolysis in order to remove fatty acids from lipid A. The resultant products retained the core region and the O-SP with its heterogeneous range of molecular weights (M_r). Conjugates were prepared by schemes that bound the O-SP at multiple sites along the O-SP and/or attempted to activate one residue of the core. The composition of these O-SP conjugates varied and there is yet no unambiguous method to reliably predict their immunogenicity and thereby their effectiveness (standardization).

Shigella dysenteriae type 1 causes endemic and epidemic dysentery worldwide. In spite of its discovery a century ago, there is still no licensed vaccine

against this bacterium [13], and it is resistant to available antibiotics in several countries19. There remains a need for a vaccine capable of eliciting a protective antibody response against *Shigella* infection.

SUMMARY OF THE INVENTION

Abbreviations: LPS: lipopolysaccharide; O-SP: O-specific polysaccharide; TT: tetanus toxoid; HSA: human serum albumin; DCC: dicyclohexyl carbodiimide; Rha*p*: rhamnopyranosyl; Gal*p*: galactopyranosyl; Glc*p*: glucopyranosyl.

It is an object of the present invention to produce an antigen based on natural, modified natural or synthetic oligo- or polysaccharides which have [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, preferably two to five [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits. It is also an object of the invention to provide molecules which are structurally related and/or antigenically similar to those oligo- and polysaccharides. Preferably, these oligo- or polysaccharides of the invention are antigenically similar to an antigenic determinant of the O-specific polysaccharide of *Shigella dysenteriae* type 1 which contains [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits. The oligo- or polysaccharides may bc conjugated to a carrier to form conjugates. These oligo- or polysaccharides and conjugates thereof are immunogenic and elicit serum antibodies that are bacteriostatic or bactericidal against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, and which are useful in the prevention and treatment of shigellosis cause by *Shigella dysenteriae*. These oligo- or polysaccharides and conjugates thereof, and the antibodies which they elicit, are also useful for studying *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, *in vitro* or its products in patients.

It is yet another object of the present invention to provide an immunogen that elicits antibodies which have bacteriostatic or bactericidal activity against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, and which react with, or bind to, one or more [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits and/or the O-specific polysaccharide of

Shigella dysenteriae type 1, wherein the immunogen is based on a natural, modified natural, or synthetic oligo- or polysaccharide containing one or more [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, or a structurally related, immunologically similar, oligo- or polysaccharide, and/or conjugate thereof.

It is yet another object of the present invention to provide antibodies which have bacteriostatic or bactericidal activity against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, and which react with, or bind to, an oligo- or polysaccharide containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits and/or the O-specific polysaccharide of *Shigella dysenteriae* type 1, wherein the antibodies are elicited by immunization with a natural, modified natural, or synthetic oligo- or polysaccharide containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, or a structurally related, immunologically similar, oligo-or polysaccharide, and/or conjugate thereof.

It is yet another object of the present invention to provide oligo- or polysaccharides or conjugates thereof which are useful as vaccines to prevent and/or treat shigellosis.

It is yet another object of the present invention to prepare antibodies for the treatment of established shigellosis. Antibodies elicited by the oligo- or polysaccharides of the invention and/or carrier conjugates thereof are useful in providing passive protection to an individual exposed to *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, to prevent, treat, or ameliorate infection and disease caused by the microorganism.

It is yet another object of the present invention to provide diagnostic tests and/or kits for shigellosis caused by *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, using one or more of the oligo- or polysaccharides, conjugates, or antibodies of the present invention.

It is yet another object of the present invention to provide an improved method for synthesizing an oligo- or polysaccharide containing one or more [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits (see

also, reference [20]).

According to the present invention, methods are provided to isolate, substantially purify and/or synthesize natural, modified natural or synthetic oligo- or polysaccharides containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, or structurally related, immunologically similar, oligo- or polysaccharides. Preferably, these oligo- and polysaccharides are structurally related and/or immunologically similar to an antigenic determinant of the O-specific polysaccharide of *Shigella dysenteriae* type 1.

Methods are also provided to conjugate the natural, modified natural, or synthetic oligo- or polysaccharides of the invention with a carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. The chemical repeating unit of the O-specific polysaccharide of *Shigella dysenteriae* type 1, with subunits labeled A-D..

Figure 2. Compounds **3**, **6**, **10**, and **11**; used to provide the monosaccharide subunits A-D. Bn = benzyl, Bz = benzoyl, CA = chloroacetyl, MBn = 4-methoxybenzyl.

Figure 3. Preparation of compound **6**.

Figure 4. Preparation of compound **10**.

Figure 5. Preparation of tetrasaccharide repeating unit **20** and iterative coupling to generate protected hexadecasaccharide **29**. TEC = 2,2,2-trichloroethoxycarbonyl.

Figure 6. Alternative to Scheme3; leading to protected hexadecasaccharide **43**.

Figure 7a. Structure of tetra- and octasaccharides **44 – 47**, having ester and/or hydrazide side chains at reducing end.

Figure 7b. Structure of dodeca-, and hexadecasaccharides **48 – 50**, having ester and/or hydrazide side chains at reducing end.

Figure 8. Preparation of 6-carbon linker molecule **52**.

Figure 9. Attachment of linker and coupling of hexadecasaccharide to protein carrier.

Figure 10. Overview of stages of glycoconjugate synthesis. CA = 3-*O*-chloroacetyl protecting group.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a synthetic, or a natural, or a modified natural, oligo- or polysaccharide containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, or a structurally and/or immunologically related antigen. The oligo- or polysaccharide containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, as a natural or synthetic product, may be covalently bound to both a saccharide carrier and a protein carrier, preferably a non-toxic non-host protein carrier, or it may be covalently bound directly to a protein carrier, preferably a non-toxic non-host protein carrier, to form a conjugate. The present invention also encompasses mixtures of the oligo- or polysaccharides and/or conjugates thereof.

Another object of this invention is to provide vaccines that will induce antibodies with bacteriostatic or bactericidal activity against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1. The vaccines of this invention contain one or more synthetic, natural, or modified natural, oligo- or polysaccharides containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, or structurally and/or immunologically related antigens, or conjugates thereof, as described above.

The saccharide-based vaccine is intended for active immunization for prevention of shigellosis and for preparation of immune antibodies as a therapy. This [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunit-based vaccine is designed to confer specific preventative immunity against infection with *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, and to induce antibodies specific to oligo- or polysaccharides containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits for therapy of shigellosis.

The oligo- and polysaccharides of the invention, and conjugates thereof, as well as the antibodies thereto, will be useful in increasing resistance to,

preventing, ameliorating, and/or treating shigellosis caused by *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, in humans.

It is expected that oligo- or polysaccharides containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, will elicit serum antibodies specific to [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→], which induce complement-dependent killing of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, when coupled with a carrier. It is also expected that these serum antibodies specific to [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] will protect against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, infection in mammals, including humans.

A number of primary uses for the compounds of this invention are envisioned. The invention is intended to be included in the routine immunization schedule of infants and children living in areas where shigellosis is endemic, and in individuals at risk for shigellosis, such as travelers to areas where shigellosis is endemic. It is also planned to be used for intervention of epidemics caused by *Shigella dysenteriae* type 1. Additionally, it is planned to be used for a multivalent vaccine for *Shigella* and other enteric pathogens for routine immunization of infants. The invention is also intended to prepare antibodies with bacteriostatic bactericidal activity to *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, for therapy of established shigellosis. The invention is also intended to provide a diagnostic test for shigellosis caused by *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1.

A synthetic saccharide-based vaccine has several important advantages over a product made from the authentic compound from *Shigella dysenteriae*, in particular by being a much better defined product whose purity can be verified by the most sophisticated analytic equipment; in addition, there are no other types of saccharides or proteins as part of the synthesized saccharide, thereby improving the safety of the vaccine.

Methods for preparing oligo- and polysaccharides

Methods for synthesizing di- to penta-, tetra-, hexa-, octa-, dodeca-

and hexadeca- saccharide fragments of the O-specific polysaccharide of *Shigella dysenteriae* type 1 have been described previously [14,15,16,18]. An improved method to synthesize oligo- or polysaccharides consisting of consecutive tetrasaccharide repeating units of the O-specific polysaccharide of *Shigella dysenteriae* type 1 is set forth in the Examples below (see also, reference [20]).

The methods of the present invention enable the iterative attachment of [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits to each other. In the examples presented herein, two, three and four [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] units are linked, but further iterations adding additional units are contemplated to be within the scope of the present invention.

Methods for preparing *Shigella dysenteriae* type 1 O-specific polysaccharide and protein conjugates thereof have been described previously [13]. High-pressure liquid chromatography of the O-specific polysaccharide through SUPEROSE 12 HR shows a minor peak of approximately 65,200 daltons and a major peak of approximately 18,700 daltons. There is also a small UV-absorbing peak of 5,700 daltons. [13]. Fragments of the O-specific polysaccharide of *Shigella dysenteriae* type 1, of varying sizes, including fragments larger than hexadecasaccharides, may be generated either enzymatically or chemically. For example, the O-specific polysaccharide preparation may be digested with an α-glucosidase or subjected to acid hydrolysis by methods known in the art. α-glucosidases are commercially available. Limited digestion, *e.g.*, digestion for varying lengths of times, may be used to generate fragments of varying lengths. Fragments of the desired size may then be separated by size chromatography. Further purification of the fragments may be conducted using methods known in the art. As with other saccharides, the molecular weight of the oligo- or polysaccharide alone and when conjugated to a carrier is related to its immunogenicity [21,22,23]. Thus, the oligo- or polysaccharide may vary in molecular weight in order to enhance its antigenicity or to enhance its immunogenicity when in a conjugate form. Preferably the synthetic oligo- or polysaccharide will have 2 to 5 synthetic [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits

linked to each other, more preferably 4 such subunits. The molecular weight of the synthetic [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ oligo- or polysaccharide may range from about 1230 daltons (where n = 2) to about 3100 daltons (where n = 5), and is preferably about 2460 daltons (where n = 4).

Definitions

"Oligosaccharide" as defined herein is a carbohydrate containing from two to sixteen monosaccharide units linked together. A "polysaccharide" as defined herein is a carbohydrate containing more than sixteen monosaccharide subunits linked together. See note [24].

As used herein, "natural" refers to a native or naturally occurring oligo- or polysaccharide which has been isolated from an organism, *e.g.*, *Shigella dysenteriae* type 1, and "modified natural" refers to native or naturally occurring polysaccharide that has been structurally altered. Such structural alterations are any alterations that render the modified polysaccharide antigenically similar to the antigenic determinant of the O-specific polysaccharide of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1. Preferably, the structural alterations substantially approximate the structure of the [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] antigenic determinant of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1.

In other words, a modified oligo- or polysaccharide of this invention is characterized by its ability to immunologically mimic the antigenic determinant of the O-specific polysaccharide of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1. Such a modified oligo- or polysaccharide is useful herein as a component in an inoculum for producing antibodies that preferably immunoreact with, or bind to, one or more of the [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits of the O-specific polysaccharide of *Shigella dysenteriae* type 1.

As used herein, the term "immunoreact" means specific binding between an antigenic determinant-containing molecule and a molecule containing an antibody combining site such as a whole antibody molecule or a portion thereof.

As used herein, the term "antibody" refers to immunoglobulin

molecules and immunologically active portions of immunoglobulin molecules. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and portions of an immunoglobulin molecule, including those portions known in the art as Fab, Fab', $F(ab')_2$ and F(v), as well as chimeric antibody molecules.

As used herein, the phrase "immunologically similar to" or "immunologically mimic" refers to the ability of an oligo- or polysaccharide of the invention to immunoreact with, or bind to, an antibody of the present invention that recognizes and binds to an oligo- or polysaccharide containing one or more [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits, and preferably recognizes and binds to a native antigenic determinant on the O-specific polysaccharide of *Shigella dysenteriae* type 1, which contains [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits.

It should be understood that an oligo- or polysaccharide of the invention need not be structurally identical to one or more of the [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits of *Shigella dysenteriae* type 1 so long as it is able to elicit antibodies that immunoreact with, or bind to, one or more [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits of the O-specific polysaccharide of *Shigella dysenteriae* type 1.

An oligo- or polysaccharide of the invention includes any substituted analog, fragment or chemical derivative (either natural or synthetic) of the O-specific polysaccharide of *Shigella dysenteriae* type 1 so long as the oligo- or polysaccharide is capable of reacting with antibodies that immunoreact with [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits of the O-specific polysaccharide of *Shigella dysenteriae* type 1 antigen. Therefore, an oligo- or polysaccharide can be subject to various changes that provide for certain advantages in its use.

The terms "substitute", "substituted" and "substitution" include the use of a chemically derivatized residue in place of a non-derivatized residue provided that the resulting modified oligo- or polysaccharide displays the requisite immunological

activity.

"Chemical derivative" refers to a modified oligo- or polysaccharide having one or more residues chemically derivatized by reaction of a functional side group. For example, one or more hydroxyl groups of the oligo- or polysaccharide may be reduced, oxidized, esterified, or etherified; or one or more acetamido groups may be hydrolyzed or replaced with other carboxamido or ureido groups. Such transformations are well-known and within the abilities of those skilled in the art of carbohydrate chemistry. Additional residues may also be added for the purpose of providing a "linker" by which the modified oligo- or polysaccharide of this invention can be conveniently affixed to a label or solid matrix or carrier. Suitable residues for providing linkers may contain amino, carboxyl, or sulfhydryl groups, for example. Labels, solid matrices and carriers that can be used with the oligo- or polysaccharide of this invention are described herein below.

In the examples presented herein, tetrasaccharide repeat units are assembled for blockwise construction of the octamer, dodecamer and hexadecamer. With a single attachment site at the reducing end, the entire chain of the oligosaccharide is available for interaction with the IgM receptor of the B-cell. The synthetic approaches exemplified herein allows the preparation of terminal saccharides of varying sequences, and allows for the incorporation of other moieties, such as fluoro, ureido, and the like , that might form hydrogen bonds with amino acid residues in the binding site of the receptor, and that could enhance the immunogenicity of the resultant conjugates.

Synthesis of oligosaccharides

One example of an overall strategy to the target polysaccharide **50** (a hexadecasaccharide fragment of the O-specific polysaccharide of *Shigella dysenteriae* type 1 having a spacer moiety as the aglycon portion, see Fig. 7b) involves the assembly of a tetrasaccharide repeating unit (**20**, Fig. 5) in a form that may be used in an iterative fashion for blockwise construction of higher oligosaccharides [24]. The present invention improves upon previous methods for synthesizing related compounds [14, 15, 18].

For the iterative building of a saccharide of this size, the repeating

tetrasaccharide frame along the polysaccharide chain corresponds to that depicted in Figure 1. Among the features of the exemplified method are 1) the tetrasaccharide building block is conveniently assembled in a stepwise scheme, 2) the Gal and the Rha synthons are installed in a prefabricated form which allows the attachment of the subsequent residue after only one deprotecting step, and 3) the use of a prefabricated synthon for the GlcN unit has no advantage with respect to the overall yield. Preferably, intermediates with multiple azide groups should be avoided because of the difficulties that may arise in their conversion to multiple acetamido functions [25].

The principles established in previous studies lead to the selection of four monosaccharide building blocks **3**, **6**, **10** (or **16**), and **11** (Figure 2) that are prepared as reported for related synthons [14, 15, 18]. The assembly of the tetrasaccharide **20** starts with the condensation of donor **11** with the rhamnose derivative **3** (Figure 5). The nonparticipating azido group of **11** assures sufficient stereocontrol to provide the disaccharide **12** in an acceptable yield. Treatment [15] of this disaccharide with HBF_4 chemoselectively removes the acetyl groups (→ **13**) while the benzoate at O-2 of the rhamnose moiety survives this treatment. Next, the triol **13** is transformed to the diacetate **15**. First the HO-4 and HO-6 groups of the GlcN unit are temporarily protected to allow a monochloroacetyl group to be installed at O-3 of this residue (→ **14**). Subsequent mild acid hydrolysis followed by *O*-acetylation and selective removal of the chloroacetyl group affords **15** in 71% overall yield from **14**. The Gal-GlcN-Rha trisaccharide may be prepared by selective activation of the methylthio galactoside **16** with MeOTf [26] in the presence of the phenylthio glycoside **15** to give stereoselective coupling, followed by conversion to the amino derivative **17**, for example by the Staudinger reaction using PPh_3 and subsequent hydrolysis, providing in this example a 58% overall yield for three steps (Figure 5).

Next, the free amino group in **17** is protected with a 2,2,2-trichloroethoxycarbonyl (TEC) group [27] followed by oxidative removal of the 4-methoxybenzyl group to afford the trisaccharide **18** in 82% overall yield for two steps. The synthesis of the fully protected tetrasaccharide repeating unit is completed

by reaction of **18** with the rhamnose donor **6** in the presence of a catalytic amount of $BF_3 \cdot Et_2O$ to afford **19** in 89% yield (Figure 5).

Experience gained with a tetrasaccharide donor similar to **19** having an anomeric phenylthio group as the leaving group indicates that while such a compound can be successfully coupled with a highly reactive primary alcohol under I_2/TfOH activation [28], the method proves unsatisfactory for the planned iterative chain elongation. More favorable results are obtained with the imidate method [29, 30]. Therefore, the phenylthio glycoside **19** is hydrolyzed with $Hg(OCOCF_3)_2$ [31] to afford the hemiacetal **20** in 93% yield, which is routinely converted to the imidate **21** as the α anomer, using CCl_3CN and a catalytic amount of DBU [30] in 85% yield.

As the first step in the iterative condensation sequence, the tetrasaccharide donor **21** is reacted with an excess of the aglycon moiety **22** under Schmidt conditions [29, 30] to provide the tetrasaccharide glycoside **23** in 68% yield (Figure 6). The next step involves the removal of the chloroacetyl group under essentially neutral conditions to afford the acceptor **24** in 96% yield for the subsequent iterations. Three repetitive cycles involving glycosylation with the tetrasaccharide donor **21** and subsequent de-chloroacetylation give the desired target compound **28** in a protected form. Using an excess of the donor, the yields in the glycosylation steps average 60% in this example, while yields in the de-chloroacetylation steps are in the 80% range. Sequential deblocking that involves

1) replacement of the TEC groups by acetyls [(i) Cd/AcOH; (ii) Ac_2O];

2) removal of the acyl protecting groups under Zemplén conditions [32]; and

3) hydrogenolytic cleavage of the benzyl groups;

affords the target hexadecasaccharide as the methyl ester, from which the hydrazide **50** (Figure 7) is obtained by treatment with hydrazine in a combined yield of 26% for four steps from **28**.

In a preferred method of synthesis, a slightly different approach to the target oligosacccharides using a tetrasaccharide donor that already carries the acetamido function may be employed (Fig. 6). In this embodiment, thiogalactoside **10** is treated with chlorine and the glycosyl chloride so obtained is allowed to react with the acceptor **15** under activation by silver trifluoromethane-sulfonate, to afford

the trisaccharide **30** in 83% yield. Next, the azido group is converted to acetamido group in a one-pot, two-step-reaction to afford **31** in 87% combined yield. Subsequently, the 4-methoxybenzyl group is removed by oxidation with ceric(IV)ammonium nitrate [33] to afford the trisaccharide acceptor **32** in 97% yield. As the final step of the tetrasaccharide assembly, condensation of the trisaccharide **32** with the rhamnosyl imidate **6** is carried out to yield the alpha-linked tetrasaccharide **33** in 92% yield. The overall yield of the **15** → **33** sequence is 61%, an improvement over the 42% yield achieved in the **15** → **19** sequence. Routine conversion of the tetrasaccharide thioglycoside to the corresponding imidate **35** was achieved via the intermediacy of the hemiacetal **34** as described above for the related compound **21**, in 67% overall yield. In reactions similar to those already presented, the spacer-linked, fully-protected tetra- (**36**), octa- (**38**), dodeca- (**40**), and hexadeca-saccharides (**42**) were prepared by iterative condensation cycles.

The use of *catalytic* amounts of the promoter $CF_3SO_3SiMe_3$ in the trichloroacetimidate coupling reactions has been found to be superior to $BF_3{\cdot}Et_2O$, and the overall yields in the condensations involving the acetamido group-containing donor **35** have been found to be generally higher than the corresponding reactions performed with *N*-TCEC-protected intermediates, establishing the utility of the acetamido-approach that is further augmented in the deprotection phase of the syntheses.

Figure 8 summarizes one approach used to construct the heterobifunctional linker derivative **52** used to attach the hydrazide-equipped saccharide to the carrier protein. The spacer molecule **22** is oxidized according to Swern [34] to yield the aldehyde "**B**" that is *in situ* protected as the acetal "**C**". Subsequently, the ester function is cleaved with LiOH to afford the free acid **51** in 78% overall yield for three steps.

The acylation of the saccharide hydrazide **50** with the linker moiety **51** may be promoted by DCC, furnishing the intermediate acetal "**D**" (Fig. 9) that is deprotected with AcOH under mild conditions to afford the aldehyde "**E**" [35]. Alternatively, the NHS ester **52** may be employed for acylation of the hydrazide (Fig. 9). Exposure of a solution of the linker-armed hexadecasaccharide and human serum

albumin to $NaCNBH_3$ at pH 7 [36] affords the corresponding glycoconjugate **53**, determined by MALDI-TOF mass spectrometry to have an average molecular weight of 93 kDa. This corresponds to an average incorporation level of 11 saccharide chains per albumin molecule, and an overall coupling efficiency of 32%. The tetra-, octa-, and the dodeca-saccharide intermediates are likewise deprotected, equipped with the linker unit, and coupled to HSA, thus affording a series of compositionally related glycoconjugates for immunological studies. The preferred embodiments of the invention are those employing the hexadecasaccharide.

Detailed procedures for carrying out the syntheses of the oligosaccharides of the invention are provided in the examples below.

Polymeric carriers

Carriers are chosen to increase the immunogenicity of the oligo- or polysaccharide and/or to raise antibodies against the carrier which are medically beneficial. Carriers that fulfill these criteria are described in the art (see, *e.g.*, references [23, 37, 38, 39, 40). A polymeric carrier can be a natural or a synthetic material containing one or more functional groups, for example primary and/or secondary amino groups, azido groups, or carboxyl groups. The carrier can be water soluble or insoluble.

Examples of water soluble peptide carriers include, but are not limited to, natural or synthetic peptides or proteins from bacteria or virus, e. g., tetanus toxin/toxoid, diphtheria toxin/toxoid, *Pseudomonas aeruginosa* exotoxin/toxoid/protein, pertussis toxin/toxoid, *Clostridium perfringens* exotoxins/toxoid, and hepatitis B surface antigen and core antigen.

Polysaccharide carriers include, but are not limited to, capsular polysaccharides from microorganisms such as the Vi capsular polysaccharide from *S. typhi*, which contains carboxyl groups and which is described in U.S. patent 5,204,098, incorporated by reference herein; *Pneumococcus* group 12 (12F and 12A) polysaccharides, which contain a terminal galactose: and *Haemophilus influenzae* type d polysaccharide, which contains an amino terminal; as well as plant, fruit, or synthetic oligo- or polysaccharides which are immunologically similar to such capsular polysaccharides, such as pectin, D-galacturonan, oligogalacturonate, or

polygalacturonate, which are described in U.S. patent 5,738,855, incorporated by reference herein.

Examples of water insoluble carriers include, but are not limited to, aminoalkyl SEPHAROSE, e. g., aminopropyl or aminohexyl SEPHAROSE (Pharmacia Inc., Piscataway, NJ), aminopropyl glass, and the like. Other carriers may be used when an amino or carboxyl group is added through covalent linkage with a linker molecule.

Methods for attaching polymeric carriers

The oligo- or polysaccharides of the invention, containing one or more [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunit, may be bound to both a carrier saccharide and a non-toxic non-host protein carrier or directly to a non-toxic non-host protein carrier to form a conjugate.

When the oligo- or polysaccharide of the invention containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits is bound to both a carrier saccharide and a non-toxic non-host protein carrier, it may be bound first to the carrier saccharide, then the [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$-carrier conjugate can be bound to the non-toxic non-host protein carrier. The complex compound would properly be described as a semi-synthetic complex molecule with three distinct domains and origins. This complex compound would first contain an oligo- or polysaccharide having [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits bound to the carrier polysaccharide, and would then contain this two-domain saccharide bound to a protein. Alternatively, the oligo- or polysaccharide of the invention containing [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits may be bound to both a carrier saccharide and a non-toxic non-host protein carrier simultaneously.

Methods for binding an oligo- or polysaccharides to a non-toxic non-host protein are well known in the art. For example, in U.S. Patent 5,204,098 and U.S. patent 5,738,855, discussed above, it is taught that an oligo- or polysaccharide containing at least one carboxyl group, through carbodiimide condensation, may be thiolated with cystamine, or aminated with adipic dihydrazide, diaminoesters,

ethylenediamine and the like. Groups which could be introduced by this method, or by other methods known in the art, include thiols, hydrazides, amines and carboxylic acids. Both the thiolated and the aminated intermediates are stable, may be freeze dried, and stored in cold. The thiolated intermediate may be reduced and covalently linked to a polymeric carrier containing a sulfhydro group, such as a 2-pyridyldithio group. The aminated intermediate may be covalently linked to a polymeric carrier containing a carboxyl group through carbodiimide condensation. (See also reference [13], where 3 different methods for conjugating the O-specific polysaccharide of *Shigella dysenteriae* type 1 to tetanus toxoid are exemplified).

The oligo- or polysaccharide can be covalently bound to a carrier with or without a linking molecule. To conjugate without a linker, for example, a carboxyl-group-containing oligo- or polysaccharide and an amino-group-containing carrier are mixed in the presence of a carboxyl activating agent, such as a carbodiimide, in a choice of solvent appropriate for both the oligo- or polysaccharide and the carrier, as is known in the art [40]. The oligo- or polysaccharide is preferably conjugated to a carrier using a linking molecule. A linker or crosslinking agent, as used in the present invention, is preferably a small linear molecule having a molecular weight of approximately <500 and is non-pyrogenic and non-toxic in the final product form, for example as disclosed in references [23, 37 - 40].

To conjugate with a linker or crosslinking agent, either or both of the oligo- or polysaccharide and the carrier may be covalently bound to a linker first. The linkers or crosslinking agents are homobifunctional or heterobifunctional molecules, *e.g.*, adipic dihydrazide, ethylenediamine, cystamine, N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl-N-(2-iodoacetyl)-β-alaninate-propionate (SIAP), succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC), 3,3'-dithiodipropionic acid, and the like. Among the class of heterobifunctional linkers area omega-hydroxy alkanoic acids.

In the examples presented herein, the linker is 6-hydroxyhexanoic acid, introduced as the methyl ester and attached via a glycosidic linkage to the oligosaccharide. Similar results would be expected with any two-to-ten-carbon omega-hydroxy alkanoic acid linker. The amino-containing linkers may be bound to

carboxyl groups of the oligo- or polysaccharide or the carrier through carbodiimide condensation. The carboxylic acid containing linkers, such as those in the examples herein, may be bound to the amino groups of the carrier through carbodiimide condensation or via N-hydroxsuccinimidyl esters. In the particular examples herein, an aldehyde linker is attached via acylation of the hydrazide groups of structures **45**, **47**, **49**, and **50**, and coupled to a carrier protein by reductive amination. The unbound materials are removed by routine physicochemical methods, such as for example gel filtration or ion exchange column chromatography, depending on the materials to be separated. The final conjugate consists of the oligo- or polysaccharide and the carrier bound through a linker. In a preferred embodiment, the polysaccharide to protein ratio (mole to mole) in the conjugate is between about 6:1 and about 12:1

Dosage for Vaccination

The present inoculum contains an effective, immunogenic amount of oligo- or polysaccharide and/or oligo- or polysaccharide-carrier conjugate of this invention. The effective amount of oligo- or polysaccharide carrier conjugate per unit dose sufficient to induce an immune response to *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, depends, among other things, on the species of mammal inoculated, the body weight of the mammal and the chosen inoculation regimen as is well known in the art. Inocula typically contain oligo- or polysaccharide carrier conjugates with concentrations of oligo- or polysaccharide of about 1 micrograms to about 100 milligrams per inoculation (dose), preferably about 3 micrograms to about 100 micrograms per dose, most preferably about 5 micrograms to 50 micrograms.

The term "unit dose" as it pertains to the inocula refers to physically discrete units suitable as unitary dosages for mammals, each unit containing a predetermined quantity of active material (oligo- or polysaccharide) calculated to produce the desired immunogenic effect in association with the required diluent.

Inocula are typically prepared as solutions in physiologically tolerable (acceptable) diluents such as water, saline, phosphate-buffered saline, or the like, to form an aqueous pharmaceutical composition.

The route of inoculation may be intramuscular, subcutaneous or the

like, which results in eliciting antibodies protective against *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1. In order to increase the antibody level, a second or booster dose may be administered approximately 4 to 6 weeks after the initial injection. Subsequent doses may be administered as indicated herein, or as desired by the practitioner. Adjuvants, such as aluminum hydroxide, may also be used.

Antibodies

An antibody of the present invention in one embodiment is characterized as comprising antibody molecules that immunoreact with: 1) oligo- or polysaccharides having one or more [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunit and 2) *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, presumably by binding to an antigenic determinant of the O-specific polysaccharide of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1.

An antibody of the present invention is typically produced by immunizing a mammal with an immunogen or vaccine containing an oligo- or polysaccharide having [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] subunits (or a structurally and/or antigenically related molecule) to induce, in the mammal, antibody molecules having immunospecificity for the immunizing oligo- or polysaccharide. The oligo- or polysaccharide or related molecule may be conjugated to a carrier. The antibody molecules may be collected from the mammal and, optionally, isolated and purified by methods known in the art.

Human or humanized monoclonal antibodies are preferred, including those made by phage display technology, by hybridomas, or by mice with human immune systems. The antibody molecules of the present invention may be polyclonal or monoclonal. Monoclonal antibodies may be produced by methods known in the art. Portions of immunoglobulin molecules, such as Fabs, may also be produced by methods known in the art.

The antibody of the present invention may be contained in blood plasma, serum, hybridoma supernatants and the like. Alternatively, the antibody of the present invention is isolated to the extent desired by well known techniques such

as, for example, ion chromatography or affinity chromatography. The antibodies may be purified so as to obtain specific classes or subclasses of antibody such as IgM, IgG, IgA, IgG_1, IgG_2, IgG_3, IgG_4 and the like. Antibody of the IgG class are preferred for purposes of passive protection.

The antibodies of the present invention have a number of diagnostic and therapeutic uses. The antibodies can be used as an *in vitro* diagnostic agent to test for the presence of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, in biological samples in standard immunoassay protocols. Such assays include, but are not limited to, agglutination assays, radioimmunoassays, enzyme-linked immunosorbent assays, fluorescence assays, Western blots and the like. In one such assay, for example, the biological sample is contacted with antibodies of the present invention and a labeled second antibody is used to detect the presence of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, or the O-specific polysaccharide antigen of *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, to which the antibodies are bound.

Such assays may be, for example, of direct format (where the labeled first antibody is reactive with the antigen), an indirect format (where a labeled second antibody is reactive with the first antibody), a competitive format (such as the addition of a labeled antigen), or a sandwich format (where both labeled and unlabelled antibody are utilized), as well as other formats described in the art.

The antibodies of the present invention are useful in prevention and treatment of infections and diseases caused by *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1.

In providing the antibodies of the present invention to a recipient mammal, preferably a human, the dosage of administered antibodies will vary depending upon such factors as the mammal's age, weight, height, sex, general medical condition, previous medical history and the like.

In general, it is desirable to provide the recipient with a dosage of antibodies which is in the range of from about 1 mg/kg to about 10 mg/kg body weight of the mammal, although a lower or higher dose may be administered.

The antibodies of the present invention are intended to be provided to

the recipient subject in an amount sufficient to prevent, lessen or attenuate the severity, extent or duration of the infection by *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1.

The administration of the agents of the invention may be for either "prophylactic" or "therapeutic" purpose. When provided prophylactically, the agents are provided in advance of any symptom. The prophylactic administration of the agent serves to prevent or ameliorate any subsequent infection. When provided therapeutically, the agent is provided at (or shortly after) the onset of a symptom of infection. The agent of the present invention may, thus, be provided either prior to the anticipated exposure to *Shigella dysenteriae*, in particular *Shigella dysenteriae* type 1, (so as to attenuate the anticipated severity, duration or extent of an infection and disease symptoms) or after the initiation of the infection.

For all therapeutic, prophylactic and diagnostic uses, the oligo- or polysaccharide of the invention, alone or linked to a carrier, as well as antibodies and other necessary reagents and appropriate devices and accessories may be provided in kit form so as to be readily available and easily used.

The immunogenicity of the synthetic conjugates of this invention in mice is greater than that of those prepared with the O-SP bound to the protein by multipoint attachment. The levels achieved by the two O-SP-HSA conjugates in mice were lower than those reported for the *S. dysenteriae* type O-SP bound to tetanus toxoid, probably due to the greater immunogenicity of the latter protein. One interpretation of the immunogenicity data in the mice is that both the density of chains and chain length are important variables. The optimal density for the octamer was 20 chains, but was 9 for both the dodecamer and the hexadecamer. Aside from the high anti-LPS level (32.6) for the hexadecamer with 9 chains (IV/43-1), there were only small differences in the GM IgG anti-LPS levels elicited by the optimal configurations of the octamer, dodecamer and hexadecamer.

The following examples are exemplary of the present processes and incorporate suitable process parameters for use herein. These parameters may be varied, however, and the following should not be deemed limiting.

EXAMPLE 1

Synthesis of oligosaccharides

The examples describe the synthesis of oligosaccharide antigens of the O-specific polysaccharide of *Shigella dysenteriae* type 1, with a heterobifunctional linker unit appended to the end of the oligosaccharide assembly for covalent attachment to human serum albumin (HSA) as a model carrier protein. The single-point attachment leaves the entire oligosaccharide chain available for interaction with B cell receptors. The average number of oligosaccharide chains attached to HSA is determined by MALDI-TOF mass spectroscopy.

General Methods. All reagent chemicals were commercial grade and used without purification. Monosaccharide starting materials were prepared by published methods. Solvents for chromatography were distilled prior to use. Anhydrous solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI). Human serum albumin (defatted) was purchased from Sigma (St. Louis, MO) and was purified by ultrafiltration through a YM10 Diaflow membrane in an ultrafiltration cell using five changes of water, followed by freeze-drying. Column chromatography was performed on silica gel 60 (0.040-0.063 mm). Melting points were taken on a capillary melting point apparatus and are uncorrected. Optical rotations were measured at 23 °C with a Perkin-Elmer Type 341 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively. Internal references: TMS (0.000 ppm for ^{1}H for solutions in $CDCl_3$), acetone (2.225 ppm for ^{1}H and 31.00 ppm for ^{13}C for solutions in D_2O), methanol (3.358 ppm for ^{1}H and 49.68 ppm for ^{13}C for solutions in D_2O), and $CDCl_3$ (77.00 ppm for ^{13}C for solutions in $CDCl_3$). Coupling constants are given in Hz. The mass spectra were recorded at the Laboratory of Analytical Chemistry, NIDDK, NIH, Bethesda, MD. Ammonia was used as the ionizing gas for the chemical ionization (CI) mass spectra. The fast atom bombardment (FAB) mass spectra were obtained using 6 keV Xe atoms to ionize samples from dithiothreitol/dithioerythritol, 3-nitrobenzyl alcohol or glycerol as the matrix. For the MALDI-TOF mass spectra the sample was dissolved in 0.1% TFA in 50% aqueous acetonitrile and applied to the target in a sinapinic acid matrix. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Abbreviations: Ac = acetyl, Bz = benzoyl, Bn = benzyl, CA = chloroacetyl, CI = chemical ionization, CSA = 10-camphorsulfonic acid, DBU = diazabicyclo[5.4.0]undec-7-ene, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMF = *N,N*-dimethylformamide, FAB = fast atom bombardment, Gal = D-galactose, GlcN = 2-amino(azido)-2-deoxy-D-glucose, MALDI-TOF = matrix-assisted laser desorption ionization time of flight, MBn = 4-methoxybenzyl, MP = 4-methoxyphenyl, MS = mass spectra/spectroscopy, NIS = N-iodosuccinimide, Ph = phenyl, PTS = 4-toluenesulfonic acid, Rha = L-rhamnose, TCEC = 2,2,2-trichloroethoxycarbonyl, TfOH = trifluoromethanesulfonic acid.

Phenyl 4-*O*-Benzyl-1-thio-α-L-rhamnopyranoside (2). To a stirred solution of **1** [41] (61 g, 206 mmol) in dry DMF (200 ml) was added at 0 °C NaH (12 g of a 60% suspension in oil) in portions. After 1 h, the mixture was treated with benzyl bromide (33 ml, 277 mmol). The mixture was stirred for 2 h then treated sequentially with MeOH and H_2O. The crystalline precipitate was isolated by filtration then treated with 90% aqueous AcOH (300 ml) at 90 °C for 3 h. The solution was concentrated. Toluene was added to and evaporated from the residue thrice. Filtration of the resulting mixture followed by washing of the solids with hexane afforded **2** (63.5 g, 89% for two steps): mp 111-113 °C; $[\alpha]_D$ -201° (c 0.9, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.45 (d, 1 H, J = 1.5), 4.75, 4.70 (2 d, 1 H each, J ~ 11), 4.21 (dq, 1 H), 4.14 (ddd, 1 H), 3.93 (ddd, 1 H), 3.42 (t, 1 H, J = 9.3), 3.14 (d, 1 H, J = 4.0), 2.80 (d, 1 H, J = 5.5), 1.34 (d, 3 H, J = 6.2); ^{13}C δ 87.4, 81.7, 72.5, 71.8, 68.6, 75.0, 17.9; CI-MS: *m/z* 364 $[(M + NH_4)^+]$. Anal. Calc'd. for $C_{19}H_{22}O_4S$: C, 65.87; H, 6.40. Found: C, 66.00; H, 6.42.

Phenyl 2-*O*-Benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (3). To a stirred solution of **2** (51.0 g, 147 mmol) in CH_2Cl_2 (100 ml) was added trimethyl orthobenzoate (30 ml, 175 mmol). The mixture was treated with a catalytic amount of CSA at 23 °C for 1 h then the solution was concentrated. To the residue was added 90% aqueous AcOH. After 10 min, the mixture was concentrated. Column chromatography of the residue (6:1 hexane-EtOAc) afforded **3** (61.2 g, 92%) as a syrup: $[\alpha]_D$ +95° (c 0.8, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.60 (dd, 1 H, J = 3.4), 5.54 (d, 1 H, J = 1.7), 4.88, 4.75 (2 d, 2 H each, J ~ 11), 4.29 (dq, 1 H), 4.20 (ddd, 1 H), 3.56

(t, 1 H, J = 9.4), 1.40 (d, 3 H, J = 6.2); ^{13}C δ 85.8, 81.6, 74.8, 70.9, 68.7, 75.1, 18.0; CI-MS: *m/z* 468 [(M + NH_4)$^+$]. Anal. Calc'd. for $C_{26}H_{26}O_5S$: C, 69.31; H, 5.82. Found: C, 69.05; H, 5.89.

Phenyl 2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside (4). To a solution of 3 (9.0 g, 20 mmol) in C_5H_5N (25 ml) at 0 °C was added chloroacetic anhydride (5.1 g, 29 mmol) under stirring. After 15 min, the solution was treated with MeOH (5 ml) and the solution was concentrated. Extractive work-up (5% aqueous HCl/$CHCl_3$) followed by drying (Na_2SO_4) and crystallization from hexane-isopropyl ether afforded 4 (9.0 g, 77%): mp 67-68 °C; $[\alpha]_D$ -201° (c 0.9, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 5.78 (dd, 1 H, J = 3.2), 5.51 (d, 1 H, J = 1.8), 5.45 (dd, 1 H), 4.74, 4.69 (2 d, 1 H each, J ~ 11), 4.40 (dq, 1 H), 3.92, 3.84 (2 d, 1 H each, J ~ 15), 3.72 (t, 1 H, J = 9.6), 1.41 (d, 3 H, J = 6.2); ^{13}C δ 166.5, 166.3, 85.6, 78.6, 74.1, 71.9, 69.1, 75.3, 17.9; CI-MS: *m/z* 436 [(M - C_7H_7 +H)$^+$]. Anal. Calc'd. for $C_{28}H_{27}ClO_6S$: C, 63.81; H, 5.16. Found: C, 63.73; H, 5.14.

2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-1-thio-α,β-L-rhamnopyranose (5). To a stirred mixture of 4 (6.5 g, 12.4 mmol) in CH_2Cl_2 (80 ml) and H_2O (2 ml) at 0 °C was added $(CF_3CO_2)_2Hg$ (6.5 g, 15.2 mmol). After 2 h, the mixture was treated with aqueous 5% KI. Concentration of the organic phase gave a semisolid that was triturated with ether and hexane. The mixture was filtered and the solids were discarded. Concentration of the mother liquor gave a syrupy residue which was purified by column chromatography (6:1 hexane-EtOAc) to afford 5 (7.7 g, 87%) as a solid: $[\alpha]_D$ +77° (c 1.0, $CHCl_3$); NMR (major anomer, $CDCl_3$): ^{1}H δ 5.47-5.23 (m, 2 H), 5.08 (br s, 1 H), 4.70, 4.64 (2 d, 1 H each), 4.15 (dq, 1 H), 3.88, 3.81 (2 d, 1 H each, J ~ 15), 3.62 (t, 1 H), 1.39 (d, 3 H); ^{13}C δ 92.2, 78.5, 73.6, 70.8, 67.8, 75.1, 40.6, 18.1; CI-MS: *m/z* 452 [(M + NH_4)$^+$]. Anal. Calc'd. for $C_{22}H_{23}ClO_7$: C, 60.76; H, 5.33. Found: C, 60.88; H, 5.38.

2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl Trichloroacetimidate (6). To a stirred solution of 5 (7.7 g, 17.7 mmol) and CCl_3CN (1.9 ml, 18.9 mmol) in CH_2Cl_2 (20 ml) at 0 °C was added DBU (500 μL, 3.3 mmol). After 1 h, the solution was concentrated. Column chromatography (8:1 hexane-EtOAc) of the residue afforded 6 (10.0 g, 98%) as a syrup: $[\alpha]_D$ +9° (c 1.2, $CHCl_3$);

NMR ($CDCl_3$): ^1H δ 6.32 (d, 1 H, J = 1.4), 5.72 (dd, 1 H), 5.52 (dd, 1 H, J = 3.1, J = 9.7), 4.74, 4.68 (2 d, 1 H each, J ~ 11 Hz), 4.13 (dq, 1 H), 3.94, 3.85 (2 d, 1 H each, J ~ 15), 3.73 (t, 1 H), 1.45 (d, 3 H); ^{13}C δ 94.8, 77.8, 73.6, 70.7, 68.7, 75.5, 40.6, 18.0; Anal. Calc'd. for $C_{24}H_{23}Cl_4NO_7$: C, 49.76; H, 4.00. Found: C, 49.64; H, 3.99.

Phenyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (8). To a stirred solution of 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranose (7) (7.5 g, 14 mmol) and (phenylthio)trimethylsilane (1.6 ml, 15.4 mmol) in dry CH_2Cl_2 at 0 °C was added $BF_3{\cdot}Et_2O$ (200 μL). The solution was allowed to reach 23 °C. After 25 min, the solution was recooled to 0 °C then was treated with Et_3N (excess). Concentration followed by crystallization from MeOH afforded **8** (7.1 g, 87%): mp 110-111 °C; $[\alpha]_D$ +12° (c 0.5, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.43 (t, 1 H, J = 9.8), 4.94, 4.67, 4.57, 4.52, 4.47, 4.40 (6 d, 1 H each), 4.62 (d, 1 H, J = 10.0), 3.95 (br d, 1 H, J = 2.8), 3.68-3.61 (m, 3 H), 3.56 (dd, 1 H); ^{13}C δ 86.7, 81.4, 77.6, 72.8, 69.7, 74.3, 73.6, 72.0, 68.8; CI-MS *m/z* 602 $[(M + NH_4)^+]$. Anal. Calc'd. for $C_{35}H_{36}O_6S$: C, 71.89; H, 6.21. Found: C, 72.00; H, 6.26.

Phenyl 3,4,6-Tri-*O*-benzyl-1-thio-β-D-galactopyranoside (9). A solution of **8** (6.8 g) in CH_2Cl_2 (50 ml) was treated with a catalytic amount of NaOMe in MeOH at 23 °C for 24 h. The solution was treated with Dowex 50 x 2 (H^+) resin, filtered and concentrated to afford **9** (6.1 g, 97%) as a crystalline material: mp 91-92 °C; $[\alpha]_D$ -10° (c 0.6, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 4.90, 4.66, 4.57, 4.50, 4.44, 4.42 (6 d, 1 H each), 4.54 (d, 1 H, J = 9.8), 4.00 (t, 1 H), 3.98 (dd, 1 H), 3.62-3.69 (m, 3 H), 3.48 (dd, 1 H, J = 9.7, J = 2.6 Hz); ^{13}C δ 88.5, 83.2, 77.5, 73.1, 69.0, 74.4, 73.6, 72.4, 68.7; CI-MS *m/z* 560 $[(M + NH_4)^+]$. Anal. Calc'd. for $C_{33}H_{34}O_5S$: C, 73.01; H, 6.31. Found: C, 72.97; H, 6.26.

Phenyl 3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (10). To a stirred solution of **9** (6.0 g, 11.1 mmol) in DMF (25 ml) was added NaH (1.0 g of a 60% suspension in oil, ~ 25 mmol) at 0 °C. After 30 min, the mixture was treated with 4-methoxybenzyl chloride (2 ml, 14.7 mmol). The stirred mixture was allowed to reach 23 °C. Extractive work-up afforded **10** (7.0 g, 95%): mp 94-95 °C; $[\alpha]_D$ +9° (c 0.9, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 4.97, 4.72, 4.70,

4.66, 4.60, 4.41 (6 d, 1 H each), 4.73 (br s, 2 H), 4.62 (d, 1 H, J = 9.6 Hz), 3.97 (dd, 1 H), 3.92 (t, 1 H), 3.79 (s, 3 H), 3.65-3.56 (m, 4 H); ^{13}C δ 87.7, 84.2, 77.3, 75.3, 74.4, 73.6 (2 C), 73.4, 72.7, 68.8, 55.3; CI-MS *m/z* 680 [(M + NH_4)$^+$]. Anal. Calc'd. for $C_{41}H_{42}O_6S$: C, 74.29; H, 6.39. Found: C, 74.26; H, 6.45.

Phenyl (3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (12). A stirred solution of **3** (61.0 g, 135 mmol), **11** (29.0 g, 83 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (16.0 g, 77 mmol), and 4Å molecular sieves (15 g) in CH_2Cl_2 (150 ml) was treated under argon with CF_3SO_3Ag (32.0 g, 125 mmol) at -20 °C. The mixture was allowed to reach 0 °C in 2 h before treatment with saturated aqueous $NaHCO_3$ solution. The mixture was filtered and the solids were washed thrice with $CHCl_3$. The organic phase was extracted with H_2O and concentrated. Column chromatography of the residue (4:1 hexane-EtOAc) afforded **12** (40.2 g, 63.5%) as an amorphous solid: [α]$_D$ +59° (c 1.2, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 5.88 (dd, 1 H), 5.57 (d, 1 H, J = 1.6), 5.44 (dd, 1 H, J = 10.5, J = 9.3), 5.36 (d, 1 H, J = 3.6), 5.00 (dd, 1 H, J = 9.6), 4.93, 4.81 (2 d, 1 H each), 4.37 (dq, 1 H), 3.72 (t, 1 H, J = 9.6), 3.31 (dd, 1 H), 2.10, 2.02, 1.89 (3 s, 3 H each), 1.45 (d, 3 H); ^{13}C δ 93.1, 86.1, 79.6, 70.1, 69.3, 68.9, 68.0, 67.5, 60.5, 75.9, 61.6, 20.7, 20.6, 20.5, 17.9; CI-MS: *m/z* 781 [(M + NH_4)$^+$]. Anal. Calc'd. for $C_{38}H_{41}N_3O_{12}S$: C, 59.76; H, 5.41. Found: C, 59.64; H, 5.37.

Phenyl (2-Azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (13). A solution of **12** (50.0 g) in MeOH (200 ml) was treated with HBF_4 (~ 54% in Et_2O, 15 ml) at 23 °C. After 3 days, the solution was concentrated to ~ 50 ml under vacuum. The residue was treated with Et_3N at 0 °C, then most of the volatiles were removed under vacuum. Column chromatography of the residue (3:2 hexane-EtOAc) afforded **13** (33 g, 79%) as a syrup: [α]$_D$ -11° (c 0.6, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 5.84 (dd, 1 H, J = 3.1), 5.53 (d, 1 H, J = 1.6), 5.20 (d, 1 H, J = 3.8), 4.81, 4.62 (2 d, 1 H each, J ~ 11), 4.30 (dq, 1 H), 4.20 (dd, 1 H), 3.12 (dd, 1 H, J = 10.3), 1.32 (d, 3 H, J = 6.2); ^{13}C δ 93.7, 85.8, 79.5, 73.2, 71.3 (2C), 69.9, 69.3, 69.0, 62.2, 75.8, 61.0, 17.8; CI-MS: *m/z* 721 [(M+H)$^+$], 632 [(M+H-N_2)$^+$], 542 [(M+H-$Me_3Si(CH_2)_2OH$)$^+$]. Anal. Calc'd. for

$C_{32}H_{35}N_3O_9S$: C, 60.27; H, 5.53. Found: C, 59.61; H, 5.60.

Phenyl [2-Azido-3-*O*-chloroacetyl-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (14). To a solution of the triol 13 (27.0 g, 42.4 mmol) and 4-methoxybenzaldehyde dimethylacetal (40 ml, 230 mmol) in CH_2Cl_2 (80 ml) was added a catalytic amount of CSA at 23 °C. After 20 min, the reaction was quenched with Et_3N. The reaction mixture was stirred with hexane for 10 min (4 x 200 ml). The hexane layer was decanted and the residue was dissolved in CH_2Cl_2 (200 ml). To this solution were added at 0 °C C_5H_5N (10 ml) and chloroacetic anhydride (16 g, 94 mmol). After 10 min, the solution was sequentially treated with MeOH and Et_3N (excess). Concentration followed by column chromatographic purification (3:1 hexane-EtOAc) of the residue gave 14 (32 g, 92%) as an amorphous solid: $[\alpha]_D$ +29° (c 1.0, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.90 (dd, 1 H, J = 3.2, J = 1.6), 5.63 (t, 1 H, J = 10.0), 5.56 (d, 1 H, J = 1.6 Hz), 5.43 (s, 1 H), *5.38* (d, 1 H, J = 3.6), 4.97, 4.70 (2 d, 1 H each, J ~ 11), 4.37 (dq, 1 H), 3.22 (dd, 1 H, J = 10.3), 1.30 (d, 3 H, J = 6.2); ^{13}C δ 101.6, 93.9, 85.9, 79.8, 78.7, 73.0, 70.6, 69.3, 68.8, 62.9, 61.0, 76.6, 68.4, 55.3, 40.5, 17.8; FAB-MS: *m/z* 660 [$(M+H)^+$]. Anal. Calc'd. for $C_{42}H_{42}ClN_3O_{11}S$: C, 60.61; H, 5.09. Found: C, 60.09; H, 5.26.

Phenyl (4,6-Di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (15). To a solution of 14 (32.0 g, 38.5 mmol) in a mixture of MeOH (200 ml) and CH_2Cl_2 (50 ml) was added at 0 °C HBF_4 (~ 54% in Et_2O, 2 ml). The solution was allowed to reach 23 °C in 30 min. The mixture was recooled to 0 °C and treated with solid $NaHCO_3$ and ice until the pH of the mixture was ~5 as indicated by pH paper. Approximately 100 ml of the volatiles were removed by distillation. The residue was diluted with CH_2Cl_2 (400 ml). The solution was extracted with H_2O (twice), dried, (Na_2SO_4) and concentrated. To the residue were added at 0 °C C_5H_5N (10 ml), Ac_2O (20 ml), and a catalytic amount of 4-dimethylaminopyridine. The solution was allowed to reach 23 °C then was treated with MeOH (~10 ml) followed by concentration. A solution of the residue in DMF (50 ml) and C_5H_5N (3 ml) was treated with thiourea (16 g, 210 mmol) at 23 °C. After 30 min, the mixture was concentrated at < 30 °C. The residue

was stirred with $CHCl_3$ (100 ml) and the mixture filtered. Concentration of the mother liquor followed by column chromatographic purification of the residue (4:1 hexane-EtOAc) gave **15** (19.7 g, 71%) as an amorphous solid: $[\alpha]_D$ +29° (c 1.0, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.87 (dd, 1 H), 5.56 (d, 1 H, *J*= 1.6), 5.29 (d, 1 H, *J*= 3.9), 4.84 (dd, 1 H, *J* = 9.3), 4.83, 4.75 (2 d, 1 H each), 4.35 (dq, 1 H), 4.26 (dd, 1 H, *J* = 3.2, *J* = 9.5), 4.18-3.93 (m, 4 H), 3.69 (t, 1 H), 3.24 (dd, 1 H *J* = 10.2), 2.11, 1.99 (2s, 3 H each), 1.41 (d, 3 H, *J* = 6.2 Hz); ^{13}C δ 93.0, 86.0, 79.7, 73.0, 70.8, 70.2, 69.2, 69.0, 67.5, 63.0, 75.5, 61.7, 20.8, 20.7, 17.9; FAB-MS: *m/z* 739 $[(M + NH_4)^+]$. Anal. Calc'd. for $C_{36}H_{39}N_3O_{11}S$: C, 59.91; H, 5.45. Found: C, 60.58; H, 5.51.

Phenyl [3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-(4,6-di-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (17). A mixture of **15** (19.2 g, 26.6 mmol), **16** (22.0 g, 36.6 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (16.0 g, 78 mmol), and 4Å molecular sieves (~ 20 g) in dry ether (200 ml) was stirred for 2 h at 23 °C followed by treatment with CF_3SO_3Me (2 ml). The mixture was stirred for 66 h during which additional amounts of CF_3SO_3Me (3 ml) were added in portions. The reaction was quenched with aqueous $NaHCO_3$ solution. Extractive work-up ($CHCl_3/H_2O$) followed by chromatographic purification (6:1 hexane-EtOAc) afforded a fraction (~ 30 g) that contained the expected trisaccharide. 12.5 g of this fraction was dissolved in CH_2Cl_2 (200 ml). To this solution was added PPh_3 (16.0 g). The solution was kept at 35-40 °C for 48 h then treated with H_2O (3 ml). The mixture was stirred at 35-40 °C for 24 h followed by concentration and column chromatographic purification of residue (2:1 hexane-EtOAc) to give **17** (7.9 g, ~ 58% from **15**) as an amorphous solid: $[\alpha]_D$ +16° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.83 (dd, 1 H), 5.55 (d, 1 H, *J*= 1.4), 5.13 (d, 1 H, *J*= 3.7), 5.06 (dd, 1 H, *J* = 9.1, *J* = 9.4), 4.88 (2H), 4.75, 4.70, 4.65, 4.58, 4.53, 4.48 (7 d, 8 H), 3.72 (s, 3 H), 2.93 (dd, 1 H, *J* = 9.5), 2.10, 1.74 (2 s, 3 H each), 1.44 (d, 3 H, *J* = 6.2); ^{13}C δ 99.2, 95.5, 86.1, 83.0, 79.8, 78.7, 76.3, 75.3, 72.8, 70.3, 69.8, 69.2, 68.8, 67.7, 75.7, 74.6, 73.3 (3 C), 68.9, 62.0, 55.5, 55.2, 20.8, 18.0; FAB-MS: *m/z*. 1248 $[(M + H)^+]$. Anal. Calc'd. for $C_{71}H_{77}NO_{17}S$: C, 68.31; H, 6.22. Found: C, 68.25; H, 6.20.

Phenyl (3,4,6-Tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (18). A stirred solution of **17** (2.8 g, 6.25 mmol) in acetone (200 ml) was treated at 0 °C sequentially with a mixture of $NaHCO_3$ (5 g) in H_2O (30 ml) and 2,2,2-trichloroethyl chloroformate (5 ml, 36.3 mmol). After 3 min, the solution was concentrated and the residue was equilibrated between $CHCl_3$ and H_2O. The organic phase was treated with DDQ (2.8 g, 12.3 mmol). After 3 h, the solution was extracted with aqueous $NaHCO_3$ and processed as usual to afford, after column chromatographic purification (3:1 hexane-EtOAc), **18** (6.66 g, 82%) as an amorphous solid: $[\alpha]_D$ +44° (c 0.8, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 6.05 (m, 1 H), 5.80 (dd, 1 H, J= 3.2), 5.46 (d, 1 H, J= 1.6), 5.30 (d, 1 H, J= 3.2), 5.11 (dd, 1 H, J ~ 9.2), 4.34 (dq, 1 H), 2.92 (dd, 1 H, J = 9.8 Hz), 2.11, 1.92 (2 s, 3 H each), 1.43 (d, 3 H, J = 6.1); ^{13}C δ 99.4, 95.8, 92.9, 86.2, 79.8, 79.5, 73.5, 73.2, 72.4, 70.6, 69.8, 69.4, 69.3, 68.9, 68.1, 75.4, 74.2, 73.9, 73.1, 68.9, 61.6, 54.2, 20.8, 20.7, 17.9. Anal. Calc'd. for $C_{66}H_{70}Cl_3NO_{18}S$: C, 60.81; H, 5.41. Found: C, 60.23; H, 5.46.

Phenyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (19). To a stirred mixture of **18** (6.4 g, 4.92 mmol), **6** (10.0 g, 17.3 mmol), 4Å molecular sieves (5 g), and CH_2Cl_2 (80 ml) was added at 0 °C $BF_3 \cdot Et_2O$ (200 μL). After 2 h, the reaction mixture was filtered and concentrated. Column chromatographic purification of the residue (5:1 hexane-EtOAc) afforded **19** (7.6 g, 89%) as an amorphous solid: $[\alpha]_D$ +40° (c 0.5, $CHCl_3$); NMR ($CDCl_3$): ^{13}C δ 170.8, 168.9, 165.8, 165.2, 165.0, 153.6, 98.1, 97.4, 92.2, 95.9, 86.1, 79.8, 79.6, 78.5, 73.5, 72.9, 72.3, 71.6, 71.3, 71.0, 70.3, 69.4, 69.3, 68.0, 67.9, 75.5, 74.9, 74.1, 73.7, 73.3, 72.5, 70.2, 62.0, 53.8, 40.6, 20.8, 17.9; FAB-MS: *m/z* 1742 [($^{12}C_{88}H_{91}{}^{35}Cl_3{}^{37}ClNO_{24}S$ + Na)$^+$]. Anal. Calc'd. for $C_{88}H_{91}Cl_4NO_{24}S \cdot H_2O$: C, 60.80; H, 5.39. Found: C, 60.29; H, 5.26.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranose (20). To a stirred mixture of **19** (400 mg, 0.23 mmol) in CH_2Cl_2 (10 ml) and H_2O (0.5 ml) at 0 °C was added $(CF_3CO_2)_2Hg$ (200 mg, 0.47 mmol). After 30 min, the mixture was processed as described for **5**. Column chromatographic purification (3:1 hexane-EtOAc) of the crude product afforded **20** (353 mg, 93%) as a solid: $[\alpha]_D$ +85° (c 0.5, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.94 (d, 1 H, $J\sim$ 10 H), 5.62, 5.58 (2 dd, 1 H each), 5.47 (dd, 1 H, J= 3.2, J= 9.6), 5.42 (br s, 1 H), 5.28, 5.13 (2 br d, 1 H each), 5.24 (t, 1 H), 2.06, 1.82 (2 s, 3 H each), 1.41, 1.27 (2 d, 3 H each, $J\sim$ 6.3); FAB-MS: *m/z* 1650 [($^{12}C_{82}H_{87}{}^{35}Cl_3{}^{37}ClNO_{25}$ + Na)$^+$]. Anal. Calc'd. for $C_{82}H_{87}Cl_4NO_{25} \cdot H_2O$: C, 59.82; H, 5.45. Found: C, 59.44; H, 5.34.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl trichloroacetimidate (21). A stirred solution of **20** (10.6 g, 6.41 mmol) in CH_2Cl_2 (100 ml) and CCl_3CN (6.0 ml, 62 mmol) at 0 °C was added DBU (500 μL, 3.3 mmol) at 23 °C. After 1 h, the solution was processed as described for **6**. Column chromatographic purification (4:1 hexane-EtOAc) of the residue afforded **21** (9.6 g, 85%) as an amorphous substance: $[\alpha]_D$ +58° (c 0.5, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 6.26 (d, 1 H, J= 2.0), 5.99 (d, 1 H, $J\sim$ 9.9 Hz), 5.74, 5.63 (2 dd, 1 H each), 5.50 (dd, 1 H, J= 3.3, $J_{3,4}$= 9.7), 5.43 (d, 1 H, J= 1.5), 5.32 (t, 1 H, $J\sim$ 9), 5.29, 5.14 (2 d, 1 H each, $J\sim$ 3.5 Hz), 2.06, 1.82 (2 s, 3 H each), 1.41, 1.27 (2 d, 3 H each, $J\sim$ 6.3). Anal. Calc'd. for $C_{84}H_{87}Cl_7NO_{25}$: C, 57.37; H, 4.99. Found: C, 57.12; H, 4.99.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-

benzyl-α-L-rhamnopyranoside (23). To a stirred mixture of the imidate **21** (2.0 g, 1.13 mmol), alcohol **22** (530 mg, 3.95 mmol), 4Å molecular sieves (3 g), and CH_2Cl_2 (20 ml) was added at 0 °C $BF_3 \cdot Et_2O$ (200 μL). After 2 h, the mixture was treated with Et_3N (excess), and filtered. The filtrate was extracted with H_2O, dried and concentrated. Column chromatographic purification (3:1 hexane-EtOAc) of the residue afforded **23** (1.52 g, 68%) as a crystalline solid: mp 109-111 °C $[\alpha]_D$ +69° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.90 (d, 1 H, $J \sim 9$), 5.62 (dd, 1 H, $J = 1.8$, $J = 3.3$), 5.52 (dd, 1 H), 5.48 (dd, 1 H, $J = 9.6$), 5.41 (d, 1 H), 5.29 (t, 1 H, $J \sim 9$), 5.23, 5.12 (2 d, 1 H each, $J \sim 3.5$ Hz), 4.99, 4.75, 4.70, 4.62, 4.55, 4.49 (6 d, 1 H each), 4.77 (br s, 1 H), 2.32 (t, 2 H), 2.06, 1.81 (2 s, 3 H each), 1.36, 1.26 (2 d, 3 H each); ^{13}C, δ 174.0, 170.7, 168.7, 165.7, 165.2 (2 C), 153.6, 98.1, 97.5, 97.4, 92.5, 95.8, 79.8. 79.6, 78.5, 73.5, 72.9, 72.2, 71.9, 71.4, 70.7, 70.3, 69.3, 68.0, 67.9, 67.8 67.6, 75.4, 74.8, 74.0, 73.7, 73.2, 72.4, 69.9, 67.9, 61.6, 54.0, 51.5, 40.5, 33.8, 28.9, 25.5, 24.5, 20.7, 20.6, 17.9, 17.8; FAB-MS: *m/z* 1702 [($^{12}C_{87}H_{98}{}^{35}Cl_2{}^{37}ClNO_{26}$ + Na)$^+$]. Anal. Calc'd. for $C_{89}H_{99}Cl_4NO_{27}$: C, 60.86; H, 5.68. Found: C, 60.76; H, 5.65.

5-(Methoxycarbonyl)pentyl (2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (24). A solution of **23** (1.52 g) in a mixture of DMF (10 ml) and C_5H_5N (1 ml) was treated with thiourea (3 g) at 23 °C. After 24 h, the mixture was processed as described for **15**. Column chromatographic purification of the residue (3:1 hexane-EtOAc) gave **24** (1.39 g, 96%) as an amorphous solid: $[\alpha]_D$ +55° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.89 (d, 1 H, $J \sim 9$ Hz), 3.66 (s, 3 H), 2.31 (t, 2 H), 2.05, 1.82 (2 s, 3 H each), 1.36, 1.29 (2 d, 3 H each); ^{13}C, δ 97.94, 97.86, 97.6, 92.5, 95.8, 81.2, 79.8 (2 C), 73.0, 72.9, 72.1, 71.8, 70.9, 70.3, 70.0, 69.4, 68.1, 67.9 (2C), 67.7, 75.5, 75.1, 74.1, 73.7, 73.3, 72.6, 70.1, 67.9, 61.6, 54.0, 51.5, 33.9, 29.0, 25.6, 24.6, 20.9, 20.8, 18.1, 18.0. Anal. Calc'd. for $C_{87}H_{98}Cl_3NO_{26}$: C, 62.20; H, 5.88. Found: C, 62.33; H, 5.96.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-

galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (25). To a stirred mixture of the imidate **21** (1.58 g, 0.90 mmol), alcohol **24** (1.04 mg, 0.62 mmol), 4Å molecular sieves (3 g), and CH_2Cl_2 (15 ml) was added at 0 °C $BF_3{\cdot}Et_2O$ (250 μL). After 30 min, the mixture was processed as described for **23**. Column chromatographic purification (3:1 hexane-EtOAc) of the residue afforded **25** (1.87 g, 91%) as an amorphous solid: $[\alpha]_D$ +79° (c 0.2, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 174.0, 170.8, 170.5, 168.8, 168.7, 165.6, 165.4, 165.2 (2 C), 164.6, 153.7, 153.6, 99.4, 98.3, 97.6, 97.5 (3 C), 92.7, 92.2, 96.0, 95.9, 75.5, 75.4, 74.8, 74.1, 74.0, 73.9, 73.7, 73.3, 72.9, 72.5, 69.6, 67.9, 61.3, 61.6, 53.9, 53.0, 51.5, 40.6, 33.9, 29.0, 25.6, 24.6, 20.8, 20.7 (2 C), 20.4, 18.0 (3 C), 17.8; FAB-MS: *m/z* 3313 $[(C_{169}H_{183}Cl_7N_2O_{50} + Na)^+]$. Anal. Calc'd. for $C_{169}H_{183}Cl_7N_2O_{50}$: C, 61.69; H, 5.61. Found: C, 61.30; H, 5.63.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (26). A solution of **25** (1.80 g, 0.55 mmol) in a mixture of DMF (10 ml) and C_5H_5N (1 ml) was treated with thiourea (4 g) at 23 °C. After 24 h the mixture was processed as described for **15**. Column chromatographic purification of the residue (3:1 hexane-EtOAc) gave **26** (1.45 g, 82%) as an amorphous solid: $[\alpha]_D$ +67° (c 0.6, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.99, 5.90 (2 d, 2 H, $J \sim 9$), 3.65 (s, 3 H), 2.29 (t, 2 H), 1.94, 1.78, 1.73, 1.70 (4 s, 3 H each), 1.36, 1.26 (2 d, 6 H each); ^{13}C, δ 174.0, 170.6, 170.4, 169.0, 168.8, 165.9, 165.3, 165.2, 164.6,

153.7, 153.6, 99.4, 97.9, 97.7, 97.6, 97.3 (2C), 92.5, 92.2, 96.0, 95.8, 75.5, 75.4, 75.0, 74.6, 74.0, 73.9, 73.8, 73.7, 73.2, 73.1, 72.8, 72.6, 70.4, 68.8, 61.3, 61.1, 53.86, 53.78, 51.4, 33.8, 28.9, 25.5, 24.6, 20.81 (2 C), 20.8, 20.4, 17.9 (4 C); FAB-MS; *m/z* 3236 [$(C_{167}H_{182}Cl_6N_2O_{49} + Na)^+$]. Anal. Calc'd. for $C_{167}H_{182}Cl_6N_2O_{49}$: C, 62.41; H, 5.71. Found: C, 62.52; H, 5.75.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-a-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (27). To a stirred mixture of the imidate **21** (1.8 g, 1.02 mmol), compound **26** (1.57 g, 0.49 mmol), 4Å molecular sieves (2 g), and CH_2Cl_2 (15 ml) was added at 0 °C $BF_3{\cdot}Et_2O$ (250 μL). After 3 h, the mixture was treated with Et_3N (excess), and filtered. The filtrate was extracted with H_2O, dried, and concentrated. Column chromatographic purification (2:1 hexane-EtOAc) of the residue afforded an amorphous substance (~ 2.1 g) that contained the expected product. A solution of this substance in DMF (10 ml) and C_5H_5N (1 ml) was treated with thiourea (1.2 g) as described for **15**. Extractive and chromatographic work-up (3:2 hexane-EtOAc) afforded **27** (1.47 g, 62% for two steps) as an amorphous solid: $[\alpha]_D$ +76° (c 0.4, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.98, 5.88, 5.87 (3 d, 1 H each, $J \sim 9$), 3.65 (s, 3 H), 2.30 (t, 2 H), 1.94, 1.76, 1.72, 1.69, 1.64, 1.60 (6 s, 3 H each), 1.35, 1.27-1.22; ^{13}C, δ 174.0, 170.6, 170.4 (2 C), 169.0, 168.8 (2 C), 165.9, 165.3 (2 C), 165.2, 164.6 (2 C), 153.7, 153.6 (2 C), 99.4, 99.3, 97.9, 97.8, 97.6, 97.3 (3 C), 97.2, 92.6, 92.3, 92.2, 96.0, 95.9, 95.8, 61.3, 61.1, 60.9, 53.8 (3 C), 51.4, 33.8, 28.9, 25.5, 24.6, 20.8-20.4, 18.0 (6 C); FAB-MS:

m/z 4770 [$(C_{247}H_{266}Cl_9N_3O_{72} + Na)^+$]. Anal. Calc'd. for $C_{247}H_{266}Cl_9N_3O_{73}$: C, 62.28; H, 5.63. Found: C, 62.36; H, 5.66.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (28). To a stirred mixture of the imidate **21** (900 mg, 0.51 mmol), compound **27** (645 mg, 0.135 mmol), 4Å molecular sieves (2 g), and CH_2Cl_2 (10 ml) was added at 0 °C $BF_3{\cdot}Et_2O$ (170 μL). After 2 h, the mixture was treated with Et_3N (excess) and filtered. The filtrate was concentrated. Column chromatography (2:1 hexane-EtOAc) of the residue afforded an inhomogeneous material (825 mg) which was re-chromatographed to afford **28** (410 mg, 48%): $[\alpha]_D$ +78° (c 0.8, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.89, 5.78 (2 H), 5.69 (3 d, 4 H, $J \sim 9$), 3.67 (s, 3 H), 2.28 (t, 2 H, $J \sim 7.5$), 1.90, 1.82, 1.70 (6 H), 1.63, 1.60, 1.55, 1.50 (7 s, 24 H). Anal. Calc'd. for $C_{329}H_{351}Cl_{13}N_4O_{96}$: C, 62.15; H, 5.56. Found: C, 61.92; H, 5.60. Subsequent elution afforded a mixture (270 mg) that contained **28** and an unidentified compound in a ~ 1:1 ratio.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-

di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (29). A solution of **28** (360 mg, 56.6 mmol) in DMF (5 ml) and C_5H_5N (500 μL) was treated with thiourea (500 mg) as described for **15**. Extractive and chromatographic work-up (1:1 hexane-EtOAc) afforded **29** (290 mg, 82%) as an amorphous solid: $[\alpha]_D$ +77° (c 0.4, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.98, 5.88, 5.86 (2H) (3 d, 4 H, *J* ~ 9), 3.65 (s, 3 H), 2.30 (t, 2 H, *J* ~ 7.4), 1.95, 1.76, 1.72, 1.70, 1.64, 1.62, 1.59, 1.58 (8 s, 3 H each); FAB-MS *m/z* 6414 $[(M + Cs)^+]$. Anal. Calc'd. for $C_{327}H_{350}Cl_{12}N_4O_{95}$: C, 62.52; H, 5.62. Found: C, 62.25; H, 5.64.

Phenyl [3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-(4,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (30). To a solution of **10** (3.31 g, 5.0 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (2.2 g, 10.7 mmol) in CH_2Cl_2 (20 ml) was added at 0 °C an excess of Cl_2 in CCl_4. After 5 min, hex-1-ene was added until the yellow color of Cl_2 disappeared. This solution was added by a syringe to a stirred mixture of **15** (2.20 g, 3.05 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (1.0 g, 4.0 mmol), 4Å molecular sieves (~ 5 g), and CH_2Cl_2 (20 ml). The mixture was cooled to -78 °C and was treated with CF_3SO_3Ag (3.2 g, 12.4 mmol). After 2 h, the mixture was processed as described for **12** to afford **30** (3.22 g, 83%) as an amorphous solid: $[\alpha]_D$ +22° (c 0.4, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.92

(dd, 1 H, J= 3.1, J= 1.7), 5.57 (d, 1 H, J= 1.6), 5.38, 5.01 (2 d, 1 H each, J~ 3.7), 5.14 (dd, 1 H, J= 9.1, J= 9.4), 3.74 (s, 3 H), 3.69 (t, 1 H, J~ 9.5), 3.57 (t, 1 H, J~ 8.6), 2.12, 1.74 (2 s, 3 H each), 1.43 (d, 3 H, J= 6.2); ^{13}C δ 113.6, 106.3, 98.8, 93.0, 86.1, 79.5, 79.0, 74.9, 74.7, 74.5, 72.7, 69.9 (2 C), 69.2, 68.9, 67.5, 62.1, 75.6, 74.8, 73.3, 73.2, 72.8, 68.4, 61.9, 55.2, 20.8, 20.7, 17.9; FAB-MS: *m/z* 1406 [(M + Cs)$^+$]. Anal. Calc'd. for $C_{71}H_{75}N_3O_{17}S$: C, 66.91; H, 5.93. Found: C, 66.76; H, 5.94.

Phenyl [3,4,6-Tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (31). To a solution of 30 (33.0 g, 25.9 mmol) in CH_2Cl_2 (270 ml) was added PPh_3 (33.0 g, 126 mmol). The solution was kept at 35-40 °C for 72 h, then treated with H_2O (10 ml). The mixture was stirred at 35-40 °C for 48 h. The organic layer was extracted with H_2O, dried (Na_2SO_4) and treated with Ac_2O (8 ml) at 23 °C for 10 min. The solution was concentrated. Column chromatographic purification of residue (2:1 hexane-EtOAc) gave **31** (29.0 g, 87%) as a crystalline solid: mp 92-94 °C; $[\alpha]_D$ +22° (c 0.4, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 5.81 (d, 1 H, J~ 9.5), 5.76 (dd, 1 H), 5.48 (d, 1 H, J= 1.7), 5.17 (dd, 1 H, J= 10.2, J= 9.1), 5.14, 4.99 (2 d, 1 H each, J~ 3.5 Hz), 4.34 (dq, 1 H), 4.24 (dd, 1 H), 3.75 (s, 3 H), 3.60 (t, 1 H, J= 9.8 Hz), 2.10, 1.71, 1.48 (3 s, 3 H each), 1.44 (d, 3 H, J= 6.3); ^{13}C δ 170.7, 169.8, 168.9, 165.1, 99.0, 93.8, 86.1, 79.9, 79.0, 75.3, 75.2, 75.0, 73.1, 70.4, 70.1, 69.8, 69.4, 68.0, 75.4, 74.5, 73.3, 73.2, 73.1, 69.2, 61.9, 55.2, 51.7, 22.6, 20.8, 20.7, 18.0; FAB-MS: *m/z* 1289 [(M + H - H_2)$^+$]. Anal. Calc'd. for $C_{73}H_{79}NO_{18}S$: C, 67.94; H, 6.17. Found: C, 68.01; H, 6.20.

Phenyl [3,4,6-Tri-*O*-benzyl-α-D-galactopyranosyl]-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (32). To a solution of **31** (22 g, 17.1 mmol) in MeCN (150 ml) and H_2O (10 ml) was added $(NH_4)_2Ce(NO_3)_6$ (11.0 g, 20 mmol) at 23 °C. After 20 min, the mixture was treated with aqueous $NaHSO_3$. Extractive workup followed by column chromatographic purification (1:1 hexane-EtOAc) of the residue afforded **32** (19.4 g, 97%) as a syrup: $[\alpha]_D$ +45° (c 0.2, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 5.82 (d, 1 H, J~ 9 Hz), 5.75 (dd, 1 H, J= 3.1), 5.48 (d,

1 H, *J*= 1.5), 5.15, 4.98 (2 d, 1 H each, *J*~ 3.4), 5.09 (dd, 1 H, *J*= 9.4, *J*= 10.3), 2.09, 1.91, 1.57 (3 s, 3 H each), 1.47 (d, 3 H, *J*~ 6.3); ^{13}C δ 170.7, 170.4, 170.0, 165.1, 100.6, 93.6, 86.1, 79.8, 79.5, 75.7, 74.1, 73.2, 70.6, 70.2, 70.1, 69.5, 68.7, 68.0, 75.3, 74.5, 73.3, 73.1, 69.3, 61.5, 51.6, 22.5, 20.8 (2 C), 18.1; FAB-MS: *m/z* 1171 [$(C_{65}H_{71}NO_{17}S + H)^+$]. Anal. Calc'd. for $C_{65}H_{71}NO_{17}S \cdot EtOAc$: C, 65.86; H, 6.33. Found: C, 65.59; H, 6.15.

Phenyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (33). To a stirred mixture of **32** (18.9 g, 16.2 mmol), **6** (32.3 g, 56 mmol), 4Å molecular sieves (10 g), and CH_2Cl_2 (360 ml) was added at 0 °C $BF_3 \cdot Et_2O$ (400 μL, 3.25 mmol). After 2 h, the reaction mixture was filtered and filtrate concentrated. Column chromatographic purification of the residue (1:1 hexane-EtOAc) afforded **33** (23.6 g, 92%) as an amorphous solid: $[\alpha]_D$ +40° (c 0.5, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 5.73 (d, 1 H, *J*~ 9), 5.71, 5.63 (2 dd, 1 H each), 5.45, 5.31 (2 d, 1 H each), 5.43 (dd, 1 H, *J*= 3.3, *J*= 9.4), 5.21 (dd, 1 H, *J*= 9, *J*= 10), 5.03, 4.92 (2 d, 1 H each, *J*~ 3.5 Hz), 2.08, 1.99, 1.57 (3 s, 3 H each), 1.39, 1.20 (2 d, 3 H each, *J*~ 6.3 Hz); ^{13}C δ 170.7, 170.0, 169.3, 165.8, 165.2, 98.8, 98.6, 94.8, 86.0, 79.6, 78.8, 78.5, 75.6, 74.5, 74.0, 73.7, 73.4, 70.8, 70.2, 69.7, 69.6, 69.5, 68.0, 67.9, 75.3, 75.1, 74.6, 73.4, 72.7, 68.5, 61.3, 51.8, 40.4, 22.7, 20.8, 20.7, 18.0, 17.7; FAB-MS: *m/z* 1586 [$(C_{87}H_{92}ClNO_{23}S + H)^+$]. Anal. Calc'd. for $C_{87}H_{92}ClNO_{23}S$: C, 65.84; H, 5.84. Found: C, 65.59; H, 5.79.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-L-rhamnopyranose (34). To a stirred mixture of **33** (23.4 mg, 14.8 mmol) in CH_2Cl_2 (200 ml) and H_2O (10 ml) at 0 °C was added $(CF_3CO_2)_2Hg$ (11.0 g, 24.6 mmol). After 40 min, the mixture was processed as described for **5.** Column chromatographic purification (first hexane, then 1:1 hexane-EtOAc) of the crude product afforded **34** (21.0 g, 97%) as an amorphous solid: NMR ($CDCl_3$): ^{1}H δ 5.79 (d, 1 H, *J*~ 9.5), 5.62, 5.48 (2 dd, 1 H each), 5.41 (dd, 1 H, *J*= 3.5, *J*= 9.4), 5.29,

5.25 (2 d, 1 H each), 5.17 (dd, 1 H, $J\sim$ 10 Hz, $J\sim$ 9 Hz), 5.04, 4.91 (2 d, 1 H each), 2.04, 1.97, 1.60 (3 s, 3 H each), 1.35, 1.17 (2 d, 3 H each, $J\sim$ 6.3); ^{13}C δ 170.9, 170.3, 169.3, 165.9, 165.5, 165.3, 98.9, 98.7, 94.6, 92.2, 79.6, 78.8, 78.6, 75.7, 74.6, 73.7 (2 C), 73.5, 70.2, 69.9, 69.8, 69.5, 68.3, 68.2, 68.0, 75.1 (2 C), 74.7, 73.4, 72.8, 68.4, 61.3, 51.7, 40.5, 22.9, 20.9, 20.7, 18.2, 17.7; FAB-MS: *m/z* 1494 [$(C_{81}H_{88}ClNO_{24} + H)^+$]. Anal. Calc'd. for $C_{81}H_{88}ClNO_{24}$: C, 65.07; H, 5.93. Found: C, 64.21; H, 5.90.

(2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl Trichloroacetimidate (35). A stirred solution of 34 (20.5 g, 13.7 mmol) in CH_2Cl_2 (200 ml) and CCl_3CN (12.0 ml, 124 mmol) at 0 °C was added DBU (500 μL, 3.3 mmol). After 1 h, the solution was processed as described for **6**. Column chromatographic purification (4:1 hexane-EtOAc) of the residue afforded **35** (21.5 g, 69%) as an amorphous substance: $[\alpha]_D$ +61° (c 0.5, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 6.29 (d, 1 H, J= 2.2), 5.7 (d, 1 H, $J\sim$ 10), 5.64, 5.62 (2 dd, 1 H each), 5.42 (dd, 1 H, J= 3.4, J= 9.4), 5.30 (d, 1 H), 5.20 (dd, 1 H, J= 10.3, J = 9.0), 5.01, 4.90 (2 d, 1 H each), 2.03, 2.00, 1.63 (3 s, 3 H each), 1.39, 1.17 (2 d, 3 H each, $J\sim$ 6.3); ^{13}C δ 170.4, 170.1, 169.3, 165.9, 165.3, 165.2, 160.2, 98.9, 98.8, 95.6, 94.1, 79.0, 78.9, 78.6, 75.9, 74.6, 73.82, 73.78, 73.6, 71.3, 70.2, 69.8, 69.5, 68.1, 68.0 (2 C)), 75.5, 75.2, 74.7, 73.4, 72.8, 68.4, 61.3), 51.8, 40.5, 23.0, 20.9, 20.8, 18.2, 17.7. Anal. Calc'd. for $C_{83}H_{88}Cl_4N_2O_{24}$: C, 60.81; H, 5.41. Found: C, 60.10; H, 5.34.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (36). To a stirred mixture of the imidate **35** (1.12 g, 0.68 mmol), alcohol **22** (450 mg, 3.1 mmol), and CH_2Cl_2 (5 ml) was added at 0 °C $CF_3SO_3SiMe_3$ (5 μL, 26 μmol). After 2 h, the mixture was treated with Et_3N (excess), extracted with H_2O, dried, and concentrated. Column chromatographic purification (3:1 hexane-EtOAc) of the

residue afforded **36** (805 mg, 73%) as an amorphous solid: $[\alpha]_D$ +71° (c 0.2, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.72 (d, 1 H, *J*~ 10), 5.63 (dd, 1 H, *J*= 1.7, *J*= 3.4), 5.42 (dd, 1 H, *J*= 3.4, *J*~ 8), 4.80 (br d, 1 H), 5.28 (d, 1 H), 5.20 (dd, 1 H, *J*= 10.5, *J*= 9.2), 4.97. 4.89 (2 d, 1 H each, *J*~ 3.5), 2.34 (t, 2 H, *J*~ 7.4), 2.04, 2.01, 1.61 (3 s, 3 H each), 1.36, 1.66 (2 d, 3 H each); ^{13}C, δ 174.0, 170.7, 170.1, 169.3, 165.8, 165.5, 165.2, 99.0, 98.9, 97.3, 95.3, 79.5, 78.7, 78.6, 76.3, 74.5, 74.2, 74.0, 73.8, 70.2, 69.7 (2 C), 69.3, 68.3, 67.9, 67.8, 75.3, 75.2, 74.7, 73.3, 72.8, 68.2, 67.8, 61.0, 51.8, 51.4, 40.4, 33.8, 28.9, 25.6, 24.5, 22.9, 21.0, 20.9, 18.1, 17.6; FAB-MS: *m/z* 1622 $[(C_{88}H_{100}ClNO_{26} + H)^+]$. Anal. Calc'd. for $C_{88}H_{100}ClNO_{26}$: C, 65.12; H, 6.21. Found: C, 64.52; H, 6.19.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (37). A solution of **36** (740 mg, 0.45 mmol) in a mixture of DMF (4 ml) and C_5H_5N (0.5 ml) was treated with thiourea (0.5 g, 6.7 mmol) at 23 °C. After 24 h, the mixture was processed as described for **15**. Column chromatographic purification of the residue (3:1 hexane-EtOAc) gave **37** (670 mg, 95%) as an amorphous solid: $[\alpha]_D$ +55° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.63 (d, 1 H, *J*~ 10), 5.45, 4.95 (2 dd, 1 H each), 5.34 (d, 1 H, *J*= 1.3), 5.16 (t, 1 H, *J*= 9.3), 4.98, 4.86 (2 d, 1 H each, *J*~ 3.5), 4.79 (d, 1 H), 2.33 (t, 2 H, *J*~ 7.5), 2.04, 1.94, 1.54 (3 s, 3 H each), 1.33, 1.23 (2 d, 3 H each); ^{13}C, δ 174.0, 170.7, 170.1, 169.0, 166.0, 165.5, 99.2, 98.2, 97.4, 94.7, 81.6, 79.6, 79.2, 76.1, 74.6, 73.6, 73.1, 72.0, 70.3, 69.9, 69.7, 69.3, 68.2 (2 C), 67.8, 75.34, 75.28, 74.7, 73.4, 72.9, 68.5, 67.9, 61.2, 51.8, 51.5, 33.9, 29.0, 25.6, 24.5, 22.8, 21.0, 20.7, 18.1, 18.0; FAB-MS: *m/z* 1546 $[(C_{86}H_{99}NO_{25} + H)^+]$.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-

galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (38). To a stirred mixture of the imidate **35** (3.8 g, 2.33 mmol), alcohol **37** (1.8 g, 1.16 mmol), 4Å molecular sieves (3 g), and CH_2Cl_2 (20 ml) was added at 0 °C $BF_3{\cdot}Et_2O$ (200 μL, 1.6 mmol). After 1 h, the mixture was processed as described for **36**. Column chromatographic purification (3:2 hexane-EtOAc) of the residue afforded **38** (1.63 g, 46%) as an amorphous solid: $[\alpha]_D$ +78° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 2.32 (t, 2 H, J ~ 7.5), 2.00, 1.93, 1.82, 1.80, 1.76, 1.47 (6 s, 3 H each), 1.7-1.52, 1.41-1.32 (2 m, 6 H), 1.38, 1.24, 1.16, 0.96 (4 d, 3 H each); ^{13}C, δ 174.0, 170.6-168.9, 165.8, 165.6, 165.26, 165.23, 165.2, 99.5, 99.3, 99.1, 98.0, 97.9, 97.5, 96.0, 93.9, 61.1, 60.9, 51.5, 51.6, 51.3, 33.9, 29.0, 25.6, 24.6, 23.1, 22.6, 21.0, 20.9, 20.7, 20.4, 18.1 (2 C), 17.9, 17.3; FAB-MS: *m/z* 3024 $[(C_{167}H_{185}ClN_2O_{48} + H)^+]$. Anal. Calc'd. for $C_{167}H_{185}ClN_2O_{48}{\cdot}H_2O$: C, 65.94; H, 6.20. Found: C, 65.50; H, 6.18.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (39). A solution of **38** (1.57 g, 0.52 mmol) in a mixture of DMF (5 ml) and C_5H_5N (1 ml) was treated with thiourea (1 g, 13 mmol) at 23 °C. After 24 h, the mixture was processed as described for **15**. Column chromatographic purification of the residue (1:1 hexane-EtOAc) gave **39** (1.47 g, 96%) as an amorphous solid: $[\alpha]_D$ +70° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.81, 5.76 (2 d, 1 H each, *J*~ 10), 5.21, 5.06 (2 dd, 1 H each), 2.32 (t, 2 H, *J*~ 7.5), 1.98, 1.84, 1.82, 1.80, 1.71, 1.48 (6 s, 3 H each), 1.38, 1.24, 1.16, 1.09 (4 d, 3 H each); ^{13}C, δ 174.0, 170.6, 170.5, 170.4, 169.7, 169.0, 168.9, 166.0, 165.5, 165.3, 165.1, 99.3, 99.1, 98.3, 97.93, 97.90, 97.5, 95.2, 93.9, 81.6, 80.1, 79.6, 79.4, 79.1, 78.9, 78.0, 76.2, 75.3, 75.2, 74.6, 73.8, 73.5, 73.2, 73.1, 72.6, 72.4, 71.0, 70.3, 69.7, 69.6, 69.4, 69.3, 68.7, 68.1, 67.9, 67.6, 67.5, 75.3, 75.2, 74.7, 74.3, 74.2, 73.5, 73.4, 73.3, 72.8,

69.6, 68.2, 67.8, 61.1, 60.0, 51.5, 51.3, 51.4, 33.8, 28.9, 25.5, 24.6), 23.0, 22.6, 20.93, 20.88, 20.7, 20.4, 18.1, 17.9, 17.8, 17.7; FAB-MS: *m/z* 2948 [$(C_{165}H_{184}N_2O_{47} + H)^+$]. Anal. Calc'd. for $C_{165}H_{184}N_2O_{47}$: C, 67.24; H, 6.29. Found: C, 66.65; H, 6.32.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (40). To a stirred mixture of the imidate **35** (2.0 g, 1.22 mmol), compound **39** (0.80 g, 0.27 mmol), and CH_2Cl_2 (5 ml) was added at 23 °C $CF_3SO_3SiMe_3$ (5 μL, 26 μmol). After 3 h, the mixture was treated with Et_3N (excess), extracted with H_2O, dried (Na_2SO_4), and concentrated. Column chromatographic purification (5:4 hexane-EtOAc) of the residue afforded **40** (860 mg, 72%) as an amorphous substance: $[\alpha]_D$ +82° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^{1}H δ 5.85-5.72 (m, 4 H), 3.66 (s, 3 H), 2.32 (t, 2 H, $J \sim 7$); ^{13}C, δ 174.0, 170.7, 170.6, 169.9, 169.7, 169.4, 168.92, 168.89, 165.8, 165.6, 165.4, 165.3, 165.2, 164.8, 99.5 (2 C), 99.4, 99.3, 99.2, 98.0, 97.9 (3 C), 97.5 (2 C), 96.1, 60.4, 51.5, 51.5, 40.6, 33.8, 29.0, 25.6, 24.6, 23.1, 22.8, 22.6, 21.0-20.4, 18.0-17.2.; FAB-MS: *m/z* 4425 [$(C_{246}H_{270}ClN_3O_{70} + H)^+$]. Anal. Calc'd. for $C_{246}H_{270}ClN_3O_{70}$: C, 66.78; H, 6.15. Found: C, 66.52; H, 6.20.

5-(Methoxycarbonyl)pentyl (2-*O*-Benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-

O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (41). A solution of 40 (820 mg, 0.18 mmol) in a mixture of DMF (5 ml) and C_5H_5N (1 ml) was treated with thiourea (1 g, 13 mmol) at 23 °C for 24 h. The usual workup followed by column chromatographic purification of the residue (5:4 hexane-EtOAc) gave **41** (720 mg, 89%) as an amorphous solid: $[\alpha]_D$ +19° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 174.0, 170.7-168.9, 166.1, 165.6, 165.5, 165.3, 165.1, 164.9, 99.3 (3C), 98.4, 98.0 (4C), 97.5, 95.4, 94.2, 94.0, 61.1, 61.0, 60.8, 51.5, 51.5, 51.3, 51.1, 33.9, 29.0, 25.6, 24.6, 23.1, 22.9, 22.7, 20.9-20.4, 18.1-17.7; FAB-MS: *m/z* 4348 $[(C_{244}H_{269}N_3O_{69} + H)^+]$. Anal. Calc'd. for $C_{244}H_{269}N_3O_{69}$: C, 67.41; H, 6.24. Found: C, 67.29; H, 6.30.

5-(Methoxycarbonyl)pentyl (2-O-Benzoyl-4-O-benzyl-3-O-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (42). To a stirred solution of the imidate **35** (780 mg, 0.48 mmol), compound **41** (720 g, 0.17 mmol) in CH_2Cl_2 (4 ml) was added at 23 °C $CF_3SO_3SiMe_3$ (5 μL, 26 μmol). After 5 h, the mixture was treated with Et_3N (excess), extracted with H_2O, and concentrated. Column chromatographic purification (5:4 hexane-EtOAc) of the residue afforded **42** (640 mg, 66%) as an amorphous substance: $[\alpha]_D$ +78° (c 0.6, $CHCl_3$); NMR ($CDCl_3$):

^{13}C, δ 170.7, 170.5 (2 C), 170.43, 170.40, 169.83, 169.80, 169.6, 169.4, 168.87, 168.83 (2 C), 165.8, 165.5, 165.4, 165.3, 165.2, 165.1, 164.81, 164.77, 99.5, 99.3, 98.1, 98.0, 97.4, 96.1, 94.32, 94.27, 93.9, 61.0, 60.8, 60.7 (2 C), 51.6, 51.3, 51.1 (2 C), 51.4, 40.4, 33.8, 28.9, 25.5, 24.5, 23.1, 22.8 (2 C), 22.6, 20.92, 20.86 (3 C), 20.7, 20.4 (3 C); FAB-MS: *m/z* 5825 [($C_{325}H_{355}ClN_4O_{92}$ + H)$^+$]. Anal. Calc'd. for $C_{325}H_{355}ClN_4O_{92}$: C, 67.02; H, 6.14. Found: C, 66.03; H, 6.09.

5-(Methoxycarbonyl)pentyl (2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (43). A solution of **42** (600 mg, 0.1 mmol) in a mixture of DMF (3 ml) and C_5H_5N (0.5 ml) was treated with thiourea (1 g, 13 mmol) at 23 °C for 26 h. The usual workup followed by column chromatographic purification of the residue (5:4 hexane-EtOAc) gave **43** (435 mg, 73%) as an amorphous solid: $[\alpha]_D$ +81° (c 0.3, $CHCl_3$); NMR ($CDCl_3$): ^1H δ 5.77, 5.75 (2 H), 5.74 (3 d, 4 H, *J* ~ 9), 2.32 (t, 2 H, *J* ~ 7.5), 1.98, 1.84, 1.81, 1.79, 1.72 (9 H), 1.71 (6 H), 1.59, 1.58, 1.47 (8 s, 36 H); ^{13}C δ 174.0, 170.7, 170.53 (2 C), 170.50, 170.3, 169.9, 169.8, 169.7, 169.0, 168.9 (3 C), 166.1, 165.5, 165.46, 165.4, 165.3, 165.1, 164.9, 164.8, 99.3 (4 C), 98.4, 98.2, 98.1 (5 C), 97.5, 95.4, 94.4, 94.3, 94.0, 61.1, 60.0 (2 C), 60.7, 51.5, 51.3, 51.2 (2 C), 51.5, 33.9, 29.7, 25.6, 24.6, 23.1, 22.9 (2 C), 22.7, 20.98, 20.94, 20.93 (2 C), 20.8, 20.4 (3 C), 18.1, 18.0 (2 C), 17.95, 17.90, 17.74 (2 C), 17.7; FAB-MS: *m/z* 5748 [($C_{323}H_{354}N_4O_{91}$ + H - H_2)$^+$], 5771 [($C_{323}H_{354}N_4O_{91}$ +

$Na)^+$]. Anal. Calc'd. for $C_{323}H_{354}N_4O_{91}$: C, 67.49; H, 6.21. Found: C, 67.35; H, 6.22. Subsequent elution afforded a fraction (85 mg) that contained **43** and a small amount of an unidentified impurity.

5-(Methoxycarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-*O*-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (44). A mixture of **24** (480 mg), Zn powder (3 g), AcOH (10 ml), and H_2O (1 ml) was stirred at 23 °C for 3 h. The mixture was filtered. Approximately half of the volatiles were removed under vacuum below 25 °C. The residue was equilibrated between $CHCl_3$ and 2 % aqueous solution of the disodium salt of ethylenediaminetetraacetic acid. The organic phase was washed with H_2O, dried, (Na_2SO_4) and concentrated. A solution of the residue in C_5H_5N (2 ml) was treated at 0 °C with Ac_2O (2 ml). After 10 min, the solution was concentrated below 25 °C. A solution of the residue in dry MeOH (25 ml) was treated at 23 °C with NaOMe (~ 100 mg). After 72 h, the solution was treated with Dowex 50 X 8-100 (H^+), then concentrated. The residue was purified through a layer of silica gel using EtOAc then 10:1 EtOAc~MeOH as eluant. The intermediate so obtained was dissolved in a mixture of EtOH (10 ml) and AcOH (1 ml) and the solution stirred under H_2 in the presence of Pd-C (10%, ~ 0.2 g) at 200 psi for 24 h. The mixture was filtered and the filtrate concentrated. A solution of the residue in H_2O was freeze-dried to afford **44** (215 mg, 83 % for four steps) as an amorphous solid: $[\alpha]_D$ +64° (c 0.3, H_2O); NMR (D_2O): ^1H δ 5.58 (d, 1 H, J= 3.4), 5.08 (d, 1 H, J= 1.2), 5.00 (d, 1 H, J= 3.2), 4.81 (d, 1 H, J= 1.3), 3.69 (s, 3 H), 3.52, 3.48 (2 t, 1 H each, J ~ 9.7), 2.41 (t, 2 H, J ~ 7.4), 2.05 (s, 3 H), 1.68-1.57, 1.43-1.28 (2 m, 12 H); ^{13}C δ 102.3, 100.3, 98.4, 94.8, 68.5, 61.4, 60.6, 52.9, 52.7, 34.4, 28.9, 25.7, 24.8, 22.8, 17.4; FAB-MS *m/z* 804 [$(C_{33}H_{57}NO_{21} + H)^+$].

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-*O*-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (45). A solution of **44** (185 mg) and NH_2NH_2 (1 ml) in dry MeOH (5 ml) was kept at 23 °C for 7 d. The solution was concentrated. H_2O was added to and distilled from the residue several times. The residue so obtained was purified by gel filtration through Biogel P-2 using 0.02 M C_5H_5N-AcOH, containing

0.01% 1,1,1-trichloro-2-methyl-2-propanol to give **45** (165 mg, 89%): $[\alpha]_D$ +73° (c 0.2, H_2O); NMR (D_2O): ^1H δ 5.60 (d, 1 H, J= 3.6), 5.08 (br s, 1 H), 5.00 (br d, 1 H, J = 3.3), 4.79 (br s, 1 H), 2.22 (t, 2 H, J ~ 7.1), 2.06 (s, 3 H), 1.67-1.56, 1.42-1.33 (2 m, 6 H), 1.31, 1.30 (2 d, 3 H each); ^{13}C δ 176.6, 175, 102.4, 100.3, 98.4, 94.9, 61.5, 60.8, 52.88, 34.4, 29.0, 25.7, 25.6, 22.8, 17.5, 17.4; FAB-MS: *m/z* 804 [$(C_{32}H_{57}N_3O_{20} + H)^+$].

5-(Methoxycarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (46). Compound **26** was treated as described for the preparation of compound **44**, to afford **46** as an amorphous material: $[\alpha]_D$ +83° (c 0.5, H_2O); ^1H NMR (D_2O) δ 5.60 (d, 2 H, J ~ 3.6), 5.14, 5.08, 5.05, 4.80 (4 br s, 1 H each), 5.04, 4.99 (2 d, 1 H each, J ~ 3.4), 3.69 (s, 3 H), 2.41 (t, J ~ 7.3), 2.06, 2.05 (2 s, 3 H each), 1.34 (3 H), 1.31 (3 H), 1.30 (6 H); FAB-MS: *m/z* 1484 [$(C_{59}H_{100}N_2O_{39} + Na)^+$].

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (47). Compound **46** was treated with NH_2NH_2 as described for the preparation of **45**, to afford **47** as an amorphous solid: $[\alpha]_D$ +81° (c 0.5, H_2O); NMR (D_2O): ^1H, δ 5.60 (d, 2 H, J ~ 3.6), 5.11 (br d, 1 H), 5.08 (br d, 1 H), 5.05 (br d, 1 H,), 5.045 (d, 1 H, J ~ 3.5), 5.00 (d, 1 H, J ~ 3.4), 4.80 (br d, 1 H), 2.28-2.18, 1.66-1.55 (2 m, 6 H), 2.06, 2.05 (2 s, 3 H each), 1.34, 1.31, 1.30 (3 d, 12 H, J ~ 6.2 Hz); ^{13}C δ 174.99, 174.94, 102.8, 102.4, 102.3, 100.3, 98.4 (2 C), 94.9 (2 C), 61.5 (2 C), 60.9, 60.7, 52.8, 34.4 (br), 28.9, 25.7, 25.6, 22.9, 17.6, 17.5 (2 C), 17.4; FAB-MS: *m/z* 1462 [$(C_{59}H_{100}N_2O_{39} + H)^+$].

5-(Methoxycarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-

(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (48). A mixture of compound **27** (440 mg, 92.3 μmol), Cd powder (1.5 g), AcOH (1.5 ml), and DMF (1.5 ml) was stirred at 23 °C for 8 h. The mixture was diluted with EtOAc, filtered, and the filtrate concentrated. Toluene was added to and evaporated from the residue to afford a syrup that was treated with C_5H_5N (5 ml) and Ac_2O (5 ml) at 23 °C for 3 h. Concentration followed by column chromatographic purification (1:1 hexane-EtOAc) afforded an intermediate (230 mg) as an amorphous substance: $[\alpha]_D$ +77° (c 0.3, $CHCl_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 99.9, 99.6, 99.3 (2 C), 98.2, 98.1 (2 C), 97.9, 97.5, 96.5, 94.2, 94.0, 61.0, 60.8, 60.7, 51.7, 51.3, 51.1, 51.5, 33.8, 29.0, 25.6, 24.6, 23.2, 22.9, 22.7, 20.9-20.4, 18.11 (2 C), 18.0, 17.9, 17.8, 17.2; FAB-MS: *m/z* 4390 $[(C_{246}H_{271}N_3O_{70} + H)^+]$. Anal. Calc'd. for $C_{246}H_{271}N_3O_{70}$: C, 67.31; H, 6.22. Found: C, 67.02; H, 6.30. This material was sequentially deacylated (NaOMe in MeOH) then hydrogenolyzed (H_2/Pd-C in EtOH-AcOH) as described for **44** to afford **48**: $[\alpha]_D$ +81° (c 0.3, H_2O); ^{13}C NMR (D_2O) δ 178.4, 175.0, 174.9 (2 C), 102.8 (2 C), 102.4, 102.3 (2 C), 100.3, 98.4 (3 C), 94.1 (3 C), 61.5 (3 C), 60.9 (2 C), 60.8, 52.9, 52.8 (3 C), 34.4, 28.9, 25.7, 24.8, 22.9 (3 C), 17.6 (2 C), 17.5 (4 C); FAB-MS: *m/z* 2141 $[(C_{85}H_{143}N_3O_{57} + Na)^+]$.

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (49).
Procedure (a): Compound **48** was treated with NH_2NH_2 as described for the preparation of **45** to afford **49** as an amorphous solid: $[\alpha]_D$ +80° (c 0.2, H_2O); NMR (D_2O): ^1H, δ 5.59 (d, 3 H, J ~ 3.7), 5.11 (d, 2 H, J ~ 1.7), 5.08 (d, 1 H, J ~ 1.7), 5.06 (d, 2 H, J ~ 1.7), 5.04 (d, 2 H, J ~ 3.4), 5.00 (d, 1 H, J ~ 3.4), 4.80 (d, 1 H, J ~ 1.7), 2.06 (s, 6 H), 2.05 (s, 3 H), 1.33 (d, 6 H, J ~ 6.1), 1.30 (d, 3 H, J ~ 6.3), 1.29 (d, 9 H,

$J \sim 6.1$); ^{13}C δ 176.6, 175.0, 179.5 (2 C), 102.8 (2 C), 102.4, 102.3 (2 C), 100.3, 98.4 (3 C), 94.9 (3 C), 68.5, 61.5 (3 C), 60.9 (2 C), 60.7, 52.8, 34.4, 28.9, 25.7, 25.6, 22.9, 17.6 (2 C), 17.5 (3 C), 17.4; FAB-MS: *m/z* 2118 $[(C_{84}H_{143}N_5O_{56} + H)^+]$.

Procedure (b): To a solution of **41** (550 mg) in MeOH (5 ml) and CH_2Cl_2 (2 ml) was added a solution of NaOMe in MeOH until the pH of the solution reached 12 as detected by a pH paper strip. After 5 d, the solution was treated with Dowex 50 X 8-100 (H^+). The mixture was filtered and the filtrate concentrated. Column chromatographic purification of the residue (first 1:1 hexane-EtOAc then EtOAc, finally 10:1 EtOAc-MeOH gave an intermediate (315 mg) which was hydrogenolyzed (H_2/PdC in EtOH-AcOH) as described for **44**. The usual work-up afforded an amorphous material, a solution of which in EtOH (3 ml) was treated with NH_2NH_2 (0.5 ml) at 23 °C for 4 d. The solution was concentrated. H_2O (3 x 30 ml) was added to and evaporated from the residue to afford a semisolid. This was dissolved in H_2O (30 ml) and the solution was freeze-dried. The latter cycle was repeated two more times to afford **49** (200 mg) that was further purified as described in Procedure (a).

5-(Hydrazinocarbonyl)pentyl α-L-Rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (50).

Procedure (a): A mixture of compound **29** (550 mg, 87.5 mmol), Cd (5 g), AcOH (5 ml), and DMF (5 ml) was stirred at 23 °C for 2 h. The mixture was diluted with EtOAc, filtered, and the filtrate concentrated. Toluene was added to and evaporated from the residue to afford a syrup which was treated with C_5H_5N (5 ml) and Ac_2O (5 ml) at 23 °C for 3 h. Concentration followed by column chromatographic purification (1:1 hexane-EtOAc) gave a homogeneous fraction {340 mg, $[\alpha]_D$ +76° (c 0.3, $CHCl_3$), ^{13}C NMR ($CDCl_3$) δ 99.9, 99.6, 99.3 (3 C), 98.2, 98.1, 98.0 (4 C), 97.5, 96.5,

94.4, 94.3, 94.0, 61.1, 60.8, 60.7 (2 C), 51.8, 51.3, 51.1 (2 C), 51.5, 33.8, 29.0, 25.6, 24.6, 23.2, 22.9 (2 C), 22.7, 18.11, 18.09, 17.99, 17.97, 17.9, 17.74, 17.70, 17.2; FAB-MS: *m/z* 5791 [$(C_{325}H_{356}N_4O_{92} + H)^+$]}. 330 mg of this fraction was treated with NaOMe in MeOH at 23 °C for 7 days. Next the solution was treated with Dowex 50 x 2 (H^+) resin, filtered and concentrated. The residue was purified through a short column of silica gel (EtOAc). The fractions containing the major product were pooled and concentrated. The residue was dissolved in a mixture of EtOH (10 ml) and AcOH (1 ml) and was stirred under hydrogen in the presence of Pd-C (10%, ~ 0.2 g) at 200 psi for 24 h. Filtration through a layer of silica gel followed by concentration afforded an amorphous substance after freeze-drying: $[a]_D$ +82° (c 0.2, H_2O); FAB-MS: *m/z* 2777 [$(C_{111}H_{186}N_4O_{75} + H)^+$]. A solution of this material in EtOH (2 ml) was treated with hydrazine (0.5 ml) at 23 °C for 3 days. The solution was concentrated. Water was added to and evaporated from the residue several times. The residue was purified through a Biogel P-4 column which was eluted with 0.02 M pyridine-AcOH to afford **50** (62 mg, 26% for 4 steps) as a white amorphous substance: $[\alpha]_D$ +78° (c 0.1, H_2O), NMR (D_2O): ^1H, δ 5.59 (d, 4 H, J ~ 3.4), 5.11 (d, 3 H, J ~ 1.6), 5.08 (d, 1 H, J ~ 1.7), 5.06 (d, 3 H, J ~ 1.6), 5.04 (d, 3 H, J ~ 3.6), 5.00 (d, 1 H, J ~ 3.5), 4.80 (d, 1 H, J ~ 1.7), 2.06 (s, 9 H), 2.05 (s, 3 H), 1.34 (d, 9 H, J ~ 6.1), 1.31 (d, 3 H, J ~ 6.3), 1.30 (d, 12 H, J ~ 6.1); ^{13}C δ 102.6 (3 C), 102.2, 102.1 (3 C), 100.3, 98.4 (4 C), 94.9 (4 C), 61.4 (4 C), 61.0 (3 C), 60.9, 52.7 (4 C), 28.8, 26.1, 25.6, 25.5, 22.8 (4 C), 17.6 (3 C), 17.4 (4 C), 17.3; FAB-MS: *m/z* 2777 [$(C_{111}H_{186}N_4O_{75} + H)^+$].

Procedure (b): Compound **43** (435 mg) was treated as described above for compound **49** (procedure (b)) to afford **50** (145 mg, 72%) as an amorphous solid.

EXAMPLE 2

Synthesis of linkers

6,6-Dimethoxyhexanoic Acid (51). To a solution of $(COCl)_2$ (5.47 ml, 62.7 mmol) in CH_2Cl_2 (50 ml) was added at -60 °C a solution of DMSO (8.90 ml, 125 mmol) in CH_2Cl_2 (20 ml) under stirring, in a period of 5 min. The solution was stirred for 2 min after the addition was complete. To this solution was added dropwise a solution of methyl 6-hydroxyhexanoate (**22**, 7.9 g, 54 mmol) in CH_2Cl_2

(40 ml). The opaque reaction mixture was stirred at -50 °C for 1 h then was allowed to reach -10 °C. After 15 min, the solution was treated with Hünig's base (30 ml), then was allowed to reach 23 °C. The solution was extracted with H_2O (3 x 50 ml), dried (Na_2SO_4), and concentrated to afford a syrup. To a solution of this material in 2,2-dimethoxypropane (60 ml) was added a catalytic amount of 4-toluenesulfonic acid. After 30 min, approx. half of the volatiles were removed. The solution so obtained was treated with Et_3N (excess) then concentrated. The residue was equilibrated between $CHCl_3$ and H_2O. The organic layer was dried (Na_2SO_4) and concentrated to give a syrup. To a solution of this material in MeOH (100 ml) was added 1 N aqueous LiOH (80 ml) at 23 °C. After 45 min, MeOH was removed by distillation. The solution was extracted with ether thrice. To the aqueous solution was added solid citric acid until the pH of the solution reached 3.5 as estimated by indicator paper. The clear solution was extracted with $CHCl_3$ (3 x 50 ml). The combined organic phase was washed with H_2O and concentrated. Column chromatographic purification of the residue (EtOAc) afforded **51** (7.4 g, 78%) as a clear liquid whose purity is estimated to be >95% (NMR): NMR ($CDCl_3$): ^1H δ 4.37 (t, 1 H, J = 5.4), 3.32 (s, 6 H), 2.37 (t, 2 H), 1.71-1.59 and 1.48-1.36 (2 m, 6 H); ^{13}C δ 179.0 (*C*=O), 104.3, 52.7, 33.8, 32.1, 24.5, 24.1; CI-MS: *m/z* 194 [$(M + NH_4)^+$].

***N*-Hydroxysuccinimide Ester of 6,6-Dimethoxyhexanoic Acid (52).** To a solution of **51** (478 mg, 2.71 mmol) in EtOAc (10 ml) were added *N*-hydroxysuccinimide (312 mg, 2.71 mmol) and 1,3-dicyclohexylcarbodiimide (614 mg, 2.98 mmol). The mixture was stirred at 23 °C for 3 h then diluted with ether (10 ml) followed by filtration. The mother liquor was concentrated to afford **52** as a syrup that was used without further purification. The purity of this material is estimated to be > 90 % (^1H NMR). During storage at 0 °C a small amount of a crystalline material separated. NMR ($CDCl_3$): ^1H δ 4.37 (t, 1 H, J ~ 5.5), 3.31 (s, 6 H), 2.85 (br s, 4 H), 2.63 (t, 2 H), 1.83-1.45 (3 m, 6 H).

EXAMPLE 3

Conjugation of oligosaccharides to protein carriers

Conjugates. Human albumin, Sigma Chemical, St. Louis, MO, was treated by diafiltration against pyrogen-free water at 4°C, and freeze-dried.

To a solution of 50 (6.5 mg, 2.3 mmol) in DMF (200 μL) was added 52 (~ 11 mg, 40 μmol). After 4 h, the solution was applied to a Biogel P-4 column (25 x 1 cm) that was eluted with H_2O. The carbohydrate-containing fractions were pooled and freeze-dried to give an amorphous solid [NMR (D_2): ^1H δ 4.50 (t, 1 H), 3.33 (2, 6 H)]. To a solution of the residue in H_2O (1 ml) was added AcOH until pH of the solution reached ~ 2.65. After 6 h, the solution was freeze-dried to provide the aldehydo-saccharide.

To the residue were added human serum albumin (1.2 mg) followed by a solution of $NaCNBH_3$ (0.21 mg) in pH 7.0 borax-phosphate buffer (30 μL). After 2 days, the solution was treated with an additional amount of $NaCNBH_3$ (0.21 mg in pH 7.0 borax-phosphate buffer). After an additional 2 days the solution was transferred to a 10 ml Amicon diafiltration apparatus equipped with a YM-10 Diaflo membrane. The solution was filtered using five changes of H_2O. A solution of the residue in H_2O was freeze-dried to give 53 as an amorphous substance that had an average MW of 120 kDa (MALDI-TOF MS).

Other conjugates reported herein were prepared in a similar fashion. Nine synthetic saccharides and two preparations of O-SP (O-SP_1 and O-SP_2) were bound to HSA. The composition of the synthetic saccharide-HSA conjugates was calculated from the spectra obtained by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry. The composition of the bacterial O-SP bound to HSA was determined by chemical analyses. These data are summarized in Table 1.

EXAMPLE 4

Generation of antibodies

Immunization. The immunogenicity of the synthetic tetra-, octa-, dodeca- and hexadeca- saccharide antigens of the O-specific polysaccharide of *Shigella dysenteriae* type 1 covalently attached to human serum albumin (HSA) as a carrier protein, in the absence of adjuvant, were determined using the methods described in reference [13] under "Immunization" and "Serology". Groups of 10 five-week-old female general purpose mice from the NIH colony were injected

subcutaneously with conjugate containing 2.5 μg of saccharide every 2 weeks and exsanguinated 7 days following the third injection. Three experiments were done.

ELISA. Immunolon 4 microtiter plates were coated with 100 μL of 10 μg/ml of *S. dysenteriae* type 1 LPS and ELISA performed as described.

Statistical analysis. Comparisons of geometric means were performed by unpaired t tests. The Statistical Analysis System (SAS) was used for all data analysis.

Results. Serum IgG anti-LPS (Table 2). All the synthetic conjugates elicited low levels of anti-LPS after the second injection that were similar between the groups and three experiments, only the geometric mean levels of IgG anti-LPS after the third injection are shown. The geometric mean (GM) anti-LPS concentrations following the third injection from the 3 experiments are shown. Neither saline nor the O-SPs alone elicited serum IgG anti-LPS (not shown). With the exception of the tetramer, O-SP_1-HSA, and O-SP_2-HSA, all the synthetic conjugates elicited anti-LPS after the second injection and a booster (statistically significant rise) after the third injection. The tetramer with 11 chains elicited a low level of anti-LPS (0.60) in the first experiment and was not evaluated further.

Excluding the tetramer, the conjugates containing the synthetic saccharides elicited higher levels of anti-LPS than the two conjugates prepared with the O-SP prepared by hydrolysis of LPS.

The levels of anti-LPS elicited by the same conjugates were similar between experiments (Table 2). The octamer with 11 chains (IV/41) elicited 2.32 to 5.02 EU (NS) in the 3 experiments. The octamer with 20 chains (IV/124 3, elicited 9.37 in Experiment 2 and 13.2 in Experiment 3 (13.2 vs. 9.37 NS). The levels elicited by the octamer with 20 chains compared to 11 chains was significant (13.2, 9.33 vs. 5.02, 4.61, 2.32, pSO.02). Similarly, the dodecamer with 9 chains (IV/54-3) elicited 17.8 and 10.1 in Experiment 2 and 6.65 and 8.93 in Experiment 3 (NS). Aside the difference between the dodecamer with 6 chains in Experiments 1 (2.92) and 2 (17.8), the levels elicited by the dodecamer with 9 chains (6.65, 8.93 and 10.1 NS) and with 24 chains 2.99 and 1.56 NS) were similar within each group and experiment. Similarly, the hexadecamer with 4 chains elicited 1.45 vs. 4.66 (NS),

with 9 chains elicited 7.06 vs. 10.5 (NS) and those with 19 chains 3.71 vs. 4.60 (NS). The hexadecamer with 9 chains elicited the highest GM level in Experiment 1 (32.6) and 7.06 in Experiment 2 and 10.5 in Experiment 3.

Table 4 shows the rank order of the 10 highest levels of anti-LPS elicited by the conjugates in the 3 experiments. The mass of CHO, determined by the product of the chain length and average number of chains per HSA, was not related to the immunogenicity of the conjugates. Most of these conjugates (8/10) had 6 to 11 saccharide chains per protein: the conjugate ranked 6^{th} was an octamer with 22 chains and rank number 10 was a hexadecamer with 4 chains.

The average level of anti-LPS elicited by the individual synthetic conjugates is depicted in Table 2. The highest average level was elicited by the hexadecamer with 9 chains (16.7) followed by the octamer with 20 chains (11.3) and then the dodecamer with 20 chains (10.9).

Table 1.

Compositional analyses of the conjugates

Conjugate code	CHO chain length	CHO chains/HSA	M_r (kD)	HSA/CHO g/g	CHO (wt %
PC-IV/33	4	11	78	7.8	13
IV/41	8	11	83	3.9	20
IV/124	8	20	98	2.1	30
IV/54-3	12	6	79	4.5	18
IV/123-3	12	9	88	3.1	24
IV/123/4	12	24	118	1.3	43
IV/43	16	4	78	5.6	15
IV/43-1	16	10	93	2.5	28
IV/122-2	16	19	120	1.2	45
O-SP_1	-30	NA*	NA	1.3	77
O-SP_2	-30	NA	NA	1.8	57

*Not applicable

Table 2.

Serum geometric mean IgG anti-LPS (ELISA U) elicited in mice [n-10] by 3 injections of synthetic *Shigella dysenteriae* type 1 O-specific polysaccharide-HSA conjugates.

Experiment	Immunogen	CHO/protein (mol/mol)	IgG anti-LPS
1	Tetramer	12	0.60
1	Octamer	11	4.62
1	Dodecamer	6	2.92
1	Hexadecamer	9	32.6
2	Octamer	11	2.32
2	Octamer	20	9.33
2	Dodecamer	9	17.8
2	dodecamer	9	10.1
2	Dodecamer	24	2.99
2	Hexadecamer	4	1.45
2	Hexadecamer	9	7.06
2	Hexadecamer	19	3.71
2	$O\text{-}SP_1$		1.45
3	Octamer	11	5.02
3	Octamer	20	13.2
3	Dodecamer	9	6.65
3	Dodecamer	9	8.93
3	Dodecamer	24	1.58
3	Hexadecamer	4	4.66
3	Hexadecamer	9	10.5
3	Hexadecamer	19	4.60
3	$O\text{-}SP_2$		0.51
3	HSA		0.20

Groups of 10 female GPM mice injected S.C. with 2.5 μg of the conjugate (based upon CHO) 3 times at 2 weeks intervals ad bled 1 week after third injection. The LPS of *S. dysenteriae* type 1 was used as the antigen for the ELISA.

Table 3.

Geometric mean serum IgG anti-LPS levels following third injection of synthetic *Shigella dysenteriae type 1* conjugates into mice [n=10], arranged by saccharide chain length*

Experiment	Immunogen	CHO/protein (mol/mol)	IgG anti-LPS
1	Octamer	11	4.62
2	Octamer	11	2.32
3	Octamer	11	5.02
3	Octamer	20	13.2
2	Octamer	20	9.33
1	Dodecamer	6	2.92
2	Dodecamer	9	17.8
3	Dodecamer	9	6.65
3	Dodecamer	9	8.93
2	Dodecamer	9	10.1
2	Dodecamer	23	2.99
3	Dodecamer	23	1.58
2	Hexadecamer	4	1.45
3	Hexadecamer	4	4.66
2	Hexadecamer	9	7.06
3	Hexadecamer	9	10.5
1	Hexadecamer	9	32.6
2	Hexadecamer	19	3.71
3	Hexadecamer	19	4.60

*Excluding the tetramer

Table 4.

Rank order of the ten highest geometric mean levels of anti-LPS IgG elicited by the synthetic conjugates

Rank order	Immunogen	Chain length	anti-LPS IgG	CHO mass*
1	Hexadecamer	9	32.6	144
2	Dodecamer	9	17.8	108
3	Octamer	20	13.2	160
4	Hexadecamer	9	10.5	144
5	Dodecamer	9	10.1	108
6	Octamer	20	9.33	160
7	Dodecamer	9	8.93	108
8	Hexadecamer	9	7.06	144
9	Dodecamer	9	6.65	96
10	Octamer	11	5.02	88

*Chain length of saccharide x number of saccharide chains

Modifications of the above described modes for carrying out the invention that are obvious to those of skill in the fields of immunology, protein chemistry, medicine, and related fields are intended to be within the scope of the following claims.

Every reference cited herein is hereby incorporated by reference in its entirety.

REFERENCES AND NOTES

1. For reviews, see:

(a) J. B. Robbins, R. Schneerson, S. Szu, V. Pozsgay, In: *Vaccinia, vaccinations and vaccinology: Jenner, Pasteur and their successors* (Ed.: S. Plotkin, B. Fantini), Elsevier, Paris, **1996**, p. 135-143.

(b) R. K. Sood, A. Fattom, V. Pavliak, R. B. Naso, *Drug Discovery Today* **1996**, *1*, 381-387.

(c) A. Fattom, *Adv. Expt. Med. Biol.* **1995**, *383*, 131-139.

(d) U. B. S. Sϕrenson, *Danish Med. Bull.* **1995**, *42*, 47-53.

(e) H. J. Jennings, R. K. Sood, In *Neoglycoconjugates. Preparation and Applications* (Eds. Y. C. Lee, R. T. Lee), Academic Press, New York, **1994**, pp. 325-371.

(f) W. Egan, *Ann.* Rep. *Med. Chem.* **1993**, *28*, 257-265.

(g) P. R. Paradiso, K. Dermody, S. Pillai, *Vaccine Research* **1993**, *2*, 239-248.

(h) H. J. Jennings, Curr. *Top. Microbiol. Immunol.* **1990**, *150*, 97-127.

2. For the development of this concept, see:

(a) K. Landsteiner, *The specificity of serological reactions,* Harvard University Press, Cambridge, **1970**.

(b) W. F. Goebel, O. T. Avery, *J. Exp. Med.* **1929**, *50*, 521-531.

3. R. Schneerson, O. Barrera, A. Sutton, J.B. Robbins, *J. Exp. Med.* **1980**, *152*, 361-376.

4. J.B. Robbins, R. Schneerson, P. Anderson, D.H. Smith, *J. Am Med. Assoc.* **1996**, *276*, 1181-1185.

5. For example:

(a) D. Cohen, S. Ashkenazi, M.S. Green, M. Gdalevich, G. Robin, R. Slepon, M. Yavzori, N. Orr, C. Block, Y. Ashkenazi, J. Schemer, D.N. Taylor, T.L. Hale, J.D. Sadoff, D. Pavliakova, R. Schneerson, J.B. Robbins, *Lancet*, **1997**, *349*, 155-0159.

(b) D. Cohen, S. Ashkenazi, M.S. Green, Y. Lerman, R. Slepon, G. Robin, N.

Orr, D.N. Taylor, J.C. Sadoff, C. Chu, J. Shiloach, R. Schneerson, J.B. Robbins, *Infect. Immun.* **1997**, *64*, 4074-4077.

6. B.A. Dmitriev, Y.A. Knirel, N.K. Kochetkov, I. Hofman, *Eur. J. Biochem.* **1976**, *66*, 559-566.

7. Robbins, J.B., and R. Schneerson. Polysaccharide-protein conjugates: A new generation of vaccines. *J. Infect. Dis.* **1990**, *161*, 821-832.

8. J.B. Robbins, C. Chu and R. Schneerson. Hypothesis for Vaccine Development: Protective Immunity to Enteric Diseases Caused by Nontyphoidal Salmonellae and Shigellae May Be Conferred by Serum IgG Antibodies to the O-Specific Polysaccharide of Their Lipopolysaccharides. *Clin. Inf. Dis.* **1992**, *15*, 346-351.

9. D. Cohen, C. Block, M.S. Green, G. Lowell, and I. Ofek, *J. Clin. Microbiol.*, **1989**, *27*, 162-167.

10. D. Cohen, M.S. Green, C. Block, T. Roauch, and I. Ofek, *J. Infect. Dis.*, **1988**, *157*, 1068-1071.

11. D.N. Taylor, A.C. Trofa, J. Sadofff, C. Chu, D. Bryla, J. Shiloach, D. Cohen, S. Ashkenazi, Y. Lerman, W. Egan, R. Schneerson, and J. B. Robbins. Synthesis, Characterization, and Clinical Evaluation of Conjugate Vaccines Composed of the O-Specific Polysaccharides of *Shigella dysenteriae* Type 1, *Shigella flexneri* Type 2a, and *Shigella sonnei* (*Plesiomonas shigelloides*) Bound to Bacterial Toxoids. *Infect. Immun.*, **1993**, *61*, 3678-3687.

12. D. Cohen, S. Ashkenazi, *et* al. Double-blind vaccine-controlled randomised efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults. *Lancet*, **1997**, *349*, 155-159.

13. C. Chu, B. Liu, D. Watson, S. Szu, D. Bryla, J. Shiloach, R. Schneerson and J.B. Robbins. Preparation, Characterization, and Immunogenicity of Conjugates Composed of the O-Specific Polysaccharide of *Shigella dysenteriae* Type 1 (Shiga's Bacillus) Bound to Tetanus Toxoid. *Infect. Immun.* **1991**, *59*, 4450-4458.

14. (a) V. Pozsgay, B. Coxon, *Carbohydr. Res.* **1995**, *277*, 171-178.

(b) V. Pozsgay, L. Pannell, *Carbohydr. Res.* **1994**, *258*, 105-122.

(c) V. Pozsgay, B. Coxon, H. Yeh, *Bioorg. Med. Chem.* **1993**, *1*, 237-257.

(d) V. Pozsgay, C.P.J. Glaudemans, J.B. Robbins, R. Schneerson, *Carbohydr. Res.* **1993**, *244*, 259-273.

(e) V. Pozsgay, C.P.J. Glaudemans, J.B. Robbins, R. Schneerson, *Tetrahedron* **1992**, *48*, 10249-10264.

15. V. Pozsgay, B. Coxon, *Carbohydr. Res.* **1994**, *257*, 189-215.

16. V. Pozsgay, C.P.J. Glaudemans, J.B. Robbins, R. Schneerson, *Biorganic & Medicinal Chem. Lett.*, **1992**, *2*, 255-260.

17. (a) V. Pavliak, J. Nashed, V. Pozsgay, P. Kovac, A. Karpas, C. Chu, R. Schneerson, J.B. Robbins, and C.P.J. Glaudemans, *J. Biol. Chem.*, **1993**, *268*, 25797-25802.

(b) C. E. Miller, A. Karpas, R. Schneerson, K. Huppi, P. Kovac, V. Pozsgay, and C. P. J. Glaudemans. 1996 Of four anti-*Shigella dysenteriae* type 1 O-polysaccharide murine antibodies, three employ V-genes that are widely divergent from those of the fourth. *Mol. Immun.* **33**:1217-1222.

18. V. Pozsgay, *J. Amer. Chem. Soc.* **1995**, *117*, 6673-6681.

19. C.P. Howson, H.V. Finegerg, B.R. Bloom, *Lancet*, **1998**, *351*, 586.

20. V. Pozsgay, B. Coxon and J.B. Robbins, Book of Abstracts, XVIII International Carbohydrate Symposium, Milan, Italy, July 21-26, 1996, p. 217, Abstract No. B0016.

21. Martin, D.G., F.G. Jarvis and K.C. Milner. 1967. Physicochemical and biological properties of sonically treated Vi antigen. J. Bacteriol. **94**:1411-1416.

22. Peeters, C.A., A-M. Tenbergen-Meekes, D.E. Evenberg, J.T. Poolman, B.J.M. Zegers and G.T. Rijkers. 1991. A comparative study of the immunogenicity of pneumococcal type 4 polysaccharide and oligosaccharide tetanus toxoid conjugates in adult mice. *J. Immunol.* **146**:4308-4314.

23. Szu, S.C., X. Li, R. Schneerson, J.H. Vickers, D. Bryla, and J.B. Robbins. 1989.

Comparative immunogenicities of Vi polysaccharide-protein conjugates composed of cholera toxin or its B subunit as a carrier bound to high- or lower-molecular-weight Vi. *Infect. Immun.* 57:3823-3827.

24. For convenience, the term "oligosaccharide" is used herein in a liberal manner to denote the saccharides described herein; this usage differs from the standard definition that oligosaccharides may contain up to and including ten monosaccharide units (Joint Commission on Biological Nomenclature, *Eur. J. Biochem.* **1982**, *126*, 433-437).

25. In unreported experiments, a pentamer of a tetrasaccharide similar to **20** was constructed, except that it contained an azido function at C-2 of the glucose units. Various attempts to convert that protected eicosasaccharide to the corresponding acetamido derivative led to inseparable, complex mixtures.

26. H. Lönn, Carbohydr. *Res.* **1985**, *139*, 105-113.

27. (a) V. Pozsgay, In: Carbohydrates, Synthetic Methods and Applications in Medicinal Chemistry, (Ed.: H. Ogura, A. Hasegawa, T. Suami), (b) Kodansha-Verlag Chemie, Tokyo-Weinheim, **1992**, p. 188-227.
(c) H. Paulsen, C. Krogmann, *Liebigs Ann. Chem.* **1989**, 1203-1213.
(d) M. Imoto, H. Yoshimura, T. Shimamoto, N. Sakaguchi, T. Shiba, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2205-2214.

28. (a) P. Konradsson, U.E. Udodong, B. Fraser-Reid, *Tetrahedron Let.* **1990**, *31*, 4313-4316.
(b) G.H. Veeneman, S. H. van Leeuwen, J.H. van Boom, *Tetrahedron Let.* **1990**, *31*, 1331-1334.

29. (a) R.R. Schmidt , J. Michel, *Angew. Chem. Int. Ed. Engl.* **1980**, 19, 731-732.
(b) *Idem.*, Angew. Chem. **1980**, *92*, 763-764.
(c) For a review, see: R.R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123.

30. M. Numata, M. Sugimoto, K. Koike, T. Ogawa, *Carbohydr. Res.* **1987**, *163*,

209-225.

31. L. Yan, D. Kahne, *J. Am. Chem. Soc.* **1996**, *118*, 9239-9248.

32. Zemplén, G., Pacsu, E., 1929 Ber. Dtsch. Chem. Ges. 62:1613-1614.

33 R. Johannson, B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1*, **1984**, 2371.

34. A.J. Mancuso, S.L. Huang, D. Swern, *J. Org. Chem.* **1978**, *43*, 2480-2482.

35. For our earlier approaches for the introduction of a terminal, aldehydo group-containing linker moiety, see:
(a) V. Pozsgay, L. Trinh, J. Shiloach, J.B. Robbins, A. Donahue Rolfe, S.B. Calderwood, *Bioconj. Chem.* **1996**, *7*, 45-55.
(b) V. Pozsgay, *Glycoconj. J.* **1993**, *10*, 133-141.

36. G.R. Gray, *Arch.* Biochem. *Biophys.* **1974**, *163*, 426-428.

37. Fattom, A., C. Lue, S.C. Szu, J. Mestecky, G. Schiffman, D. A. Bryla, W.F. Vann, D. Watson, L.M. Kimzey, J.B. Robbins, and R. Schneerson. Serum antibody response in adult volunteers elicited by injection of Streptococcus pneumoniae type 12F polysaccharide alone or conjugated to diphtheria toxoid. *Infect. Immun.* **1990**, *58*, 2309-2312.

38. Devi, S.J., J.B. Robbins and R. Schneerson. 1992. Antibodies to poly[(2→8)-α-N-acetylneuraminic acid] are elicited by immunization of mice with *Escherichia coli* K92 conjugates: Potential vaccines for groups B and C meningococci and *E. coli*. *Proc. Natl. Acad. Sci. USA* 88:7175-7179, 1991.

39. Szu, S.C., X. Li, A.L. Stone, and J.B. Robbins. 1991. Relation between structure and immunologic properties of the Vi capsular polysaccharide. *Infect. Immun.* 59:4555-4561.

40. Szu, S.C., A.L. Stone, J.D. Robbins, R. Schneerson, and J.B. Robbins. 1987. Vi capsular polysaccharide-protein conjugates for prevention of typhoid fever. *J. Exp. Med.* 166:1510-1524.

41. V. Pozsgay, L. Pannell, *Carbohydr. Res.* **1994**, *258*, 105-122.

CLAIMS

We claim:

1. A conjugate molecule comprising a saccharide consisting of [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits, wherein n is 1 to 5, covalently bound to a protein.

2. The conjugate molecule of claim 1 wherein n is 4.

3. The conjugate molecule of claim 1 or 2 wherein the protein is covalently bound to the saccharide by reductive amination.

4. The conjugate molecule of claim 1 or 2 wherein the protein is selected from the group consisting of tetanus toxin/toxoid, diphtheria toxin/toxoid, *Pseudomonas aeruginosa* exotoxin/toxoid/protein, pertussis toxin/toxoid, *Clostridium perfringens* exotoxins/toxoid, and hepatitis B surface antigen and core antigen.

5. The conjugate molecule of claim 4 wherein the protein is tetanus toxoid.

6. The conjugate molecule of claim 1 or 2 wherein the polysaccharide to protein ratio (mole to mole) is between about 6:1 and about 12:1.

7. A pharmaceutical composition comprising a conjugate molecule comprising a saccharide consisting of [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits, wherein n is 1 to 5, covalently bound to a protein, in a physiologically acceptable carrier.

8. A pharmaceutical composition of claim 7 wherein n is 4.

9. The pharmaceutical composition of claim 7 or 8 wherein the protein is covalently bound to the saccharide by reductive amination.

10. The pharmaceutical composition of claim 7 or 8 wherein the protein is selected from the group consisting of tetanus toxin/toxoid, diphtheria toxin/toxoid, *Pseudomonas aeruginosa* exotoxin/toxoid/protein, pertussis toxin/toxoid, *Clostridium perfringens* exotoxins/toxoid, and hepatitis B surface antigen and core antigen.

11. The pharmaceutical composition of claim 10 wherein the protein is tetanus toxoid.

12. The pharmaceutical composition of claim 7 or 8 wherein the polysaccharide to protein ratio (mole to mole) is between about 6:1 and about 12:1.

13. A vaccine composition comprising, in a physiologically acceptable carrier, a conjugate molecule comprising a saccharide consisting of [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits, wherein n is 1 to 5, covalently bound to a protein.

14. The vaccine composition of claim 13 wherein n is 4.

15. The vaccine composition of claim 13 or 14 wherein the protein is covalently bound to the saccharide by reductive amination.

16. The vaccine composition of claim 13 or 14 wherein the protein is selected from the group consisting of tetanus toxin/toxoid, diphtheria toxin/toxoid, *Pseudomonas aeruginosa* exotoxin/toxoid/protein, pertussis toxin/toxoid, *Clostridium perfringens* exotoxins/toxoid, and hepatitis B surface antigen and core antigen.

17. The vaccine composition of claim 16 wherein the protein is tetanus toxoid.

18. The vaccine composition of claim 13 or 14 wherein the polysaccharide to protein ratio (mole to mole) is between about 6:1 and about 12:1.

19. A method of inducing serum antibodies that are bacteriostatic, or bactericidal to *Shigella dysenteriae* type 1 in the presence of complement and human peripheral blood polymorphonuclear leukocytes, comprising administering to a mammal, in a physiologically acceptable carrier, an immunologically sufficient amount of a conjugate molecule comprising a saccharide consisting of [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits, wherein n is 1 to 5, covalently bound to a protein, to elicit said antibodies.

20. The method of claim 19 wherein n is 4.

21. The method of claim 19 or 20 wherein said conjugate molecule is administered at a dose of about 5 micrograms to about 50 micrograms of saccharide.

22. The method of claim 20 or 21 wherein the antibodies protect the mammal against infection by *Shigella dysenteriae* type 1.

23. The method of claim 19 or 20 wherein the protein is covalently bound to the saccharide by reductive amination.

24. The method of claim 19 or 20 wherein the protein is selected from the group consisting of tetanus toxin/toxoid, diphtheria toxin/toxoid, *Pseudomonas aeruginosa* exotoxin/toxoid/protein, pertussis toxin/toxoid, *Clostridium perfringens*

exotoxins/toxoid, and hepatitis B surface antigen and core antigen.

25. The method of claim 24 wherein the protein is tetanus toxoid.

26. The method of claim 19 or 20 wherein the polysaccharide to protein ratio (mole to mole) is between about 6:1 and about 12:1.

27. A method of passively immunizing a mammal against *Shigella dysenteriae* type 1 comprising: administering *in vivo* an immunologically sufficient amount of an isolated antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof has been elicited by immunizing using a conjugate molecule comprising a saccharide consisting of four [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits, wherein n is 1 to 5, covalently bound to a protein, in a physiologically acceptable carrier.

28. The method of claim 27 wherein n is 4.

29. The method of claim 27 or 28 wherein antibody or antigen binding fragment is administered at a dose in the range of from about 1 mg/kg to about 10 mg/kg body weight of the mammal.

30. The method of claim 27 wherein the mammal is a human.

31. The method of claim 28 wherein the mammal is a human.

32. A method for vaccinating against *Shigella dysenteriae* type 1 infection in a human comprising administering to the human an immunizing amount of a composition comprising a conjugate molecule comprising a saccharide consisting of [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits, wherein n is 1 to 5, covalently bound to a protein, in a pharmaceutically acceptable carrier.

33. The method of claim 32 wherein n is 4.

34. An isolated antibody elicited by immunizing using a conjugate molecule comprising a saccharide consisting of four [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ subunits, wherein n is 1 to 5, covalently bound to a protein, in a physiologically acceptable carrier.

35. An antibody of claim 34 wherein n is 4.

36. A process for preparing an octasaccharide, dodecasaccharide, hexadecasaccharide, or eicosasaccharide, having repeating [3)-α-L-Rha*p*-(1→2)-α-D-

Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] units, comprising the steps of:

(a) providing monosaccharide derivatives **3, 6, 11, and 16;**

(b) glycosylating **3** with **11** to provide disaccharide **12;**

(c) selectively removing the acetyl groups from **12** to obtain the triol **13;**

(d) protecting the 4-OH and 6-OH groups of the GlcN unit of **13** by formation of a 4-methoxybenzaldehyde cyclic acetal;

(e) preparing the chloroacetic ester of the 5-OH group of the GlcN unit to provide **14;**

(f) removing the cyclic acetal protecting group from the 4-OH and 6-OH groups of the GlcN unit of **14;**

(g) acetylating the 4-OH and 6-OH groups of the GlcN unit;

(h) removing the chloroacetyl protecting group from the 5-OH group of the GlcN unit, to provide the disaccharide **15;**

(i) glycosylating **15** with **16** to provide a trisaccharide;

(j) reducing the azido group of the trisaccharide to provide **17;**

(k) acylating the amino group of **17** with either a 2,2,2-trichloroethoxycarbonyl group or an acetyl group;

(l) removing the 4-methoxybenzyl group from the Gal unit to provide trisaccharide **18;**

(m) glycosylating **18** with **6** to provide the tetrasaccharide **19;**

(n) hydrolyzing the thioglycoside to provide the hemiacetal **20;**

(o) converting the hemiacetal **20** to the trichloroacetimidate **21;**

(p) glycosylating methyl 6-hydroxyhexanoic acid **22** with **21** to provide the glycosylated ester **23;**

(q) removing the chloroacetyl group of **23** to provide **24;**

(r) glycosylating the resulting product with **21;**

(s) removing the chloroacetyl group;

(t) repeating steps (r) and (s) until the desired number of tetrasaccharide units have been introduced;

(u) removing trichloroethoxycarbonyl groups, if present, from the GlcN units;

(v) acetylating any resulting amino groups on the GlcN units;

(w) hydrolyzing all of the O-acetyl groups; and

(x) removing all the benzyl groups.

37. A process for preparing an octasaccharide, dodecasaccharide, hexadecasaccharide, or eicosasaccharide, having repeating [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] units, comprising the steps of

(a) providing the tetrasaccharide **33**;

(b) hydrolyzing the thioglycoside to provide the hemiacetal **34**;

(c) converting the hemiacetal **34** to the trichloroacetimidate **35**;

(d) glycosylating methyl 6-hydroxyhexanoate with **35** to provide the glycosylated ester **36**;

(e) removing the chloroacetyl group from **36** to provide **37**;

(f) glycosylating the resulting product with **35**;

(g) removing the chloroacetyl group;

(h) repeating steps (f) and (g) until the desired number of tetrasaccharide units have been introduced;

(i) hydrolyzing all of the O-acetyl groups; and

(j) removing all the benzyl groups.

38. A process of preparing an octasaccharide, dodecasaccharide, hexadecasaccharide, or eicosasaccharide protein glycoconjugate, having repeating [3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→] units covalently bound to a protein comprising

(a) converting the ester group of the product prepared according to either one of claims 36 and 37 to the corresponding hydrazide;

(b) acylating the hydrazide with the linker moiety **51** to provide the intermediate acetal, and deprotecting with acid to afford the aldehydo-saccaride; and

(c) exposing a solution of the aldehydo-saccharide and protein to $NaCNBH_3$ at pH 7 to afford the glycoconjugate.

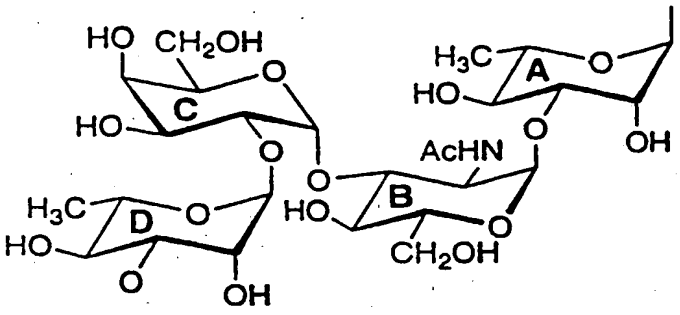
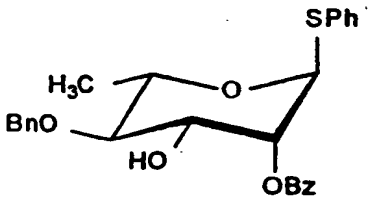
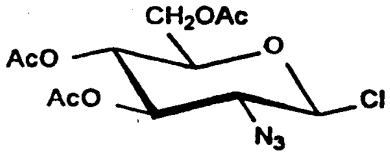


FIG. 1



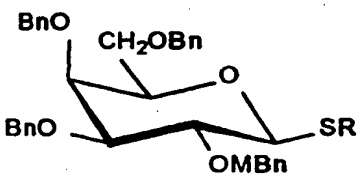
3

Residue **A**



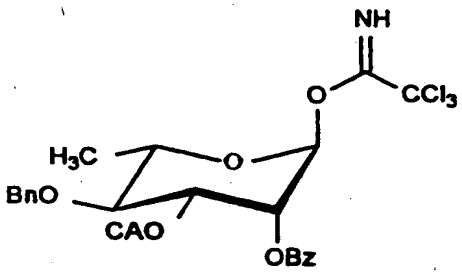
11

Residue **B**



10, R = Ph
16, R = Me

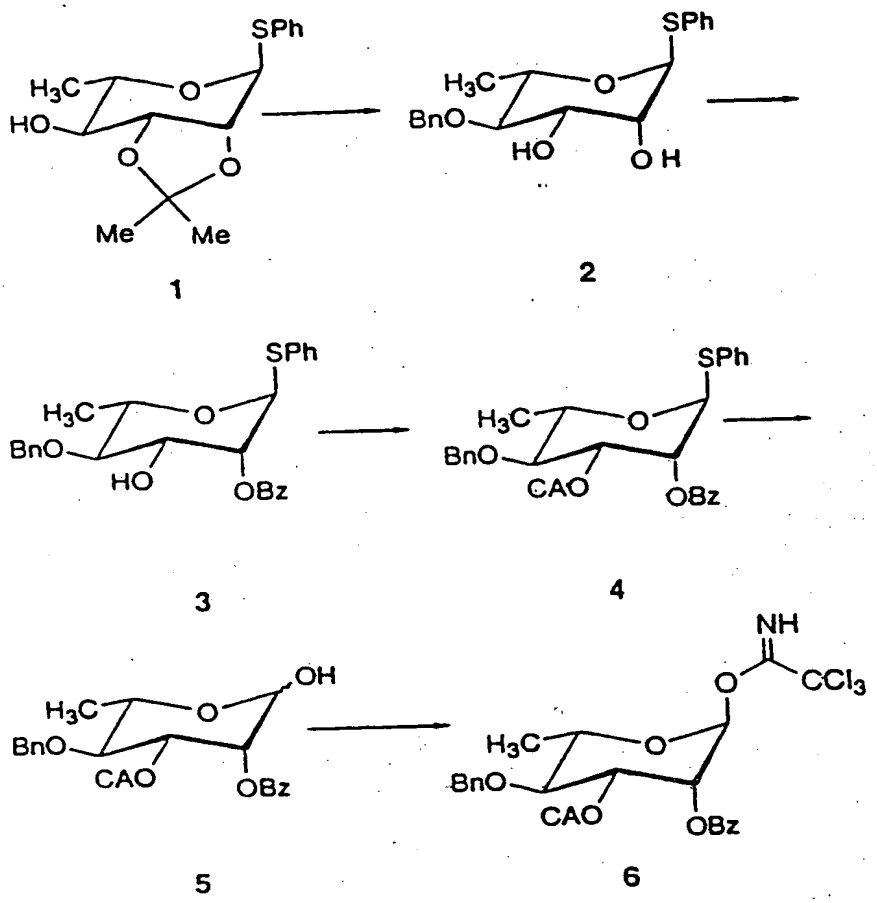
Residue **C**



6

Residue **D**

Fig. 2


SPh
H3C
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HO
O
O
Me
Me
1
SPh
H3C
O
BnO
HO
OH
2
SPh
H3C
O
BnO
HO
OBz
3
SPh
H3C
O
BnO
CAO
OBz
4
OH
H3C
O
BnO
CAO
OBz
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O
BnO
CAO
OBz
6

Fig. 3

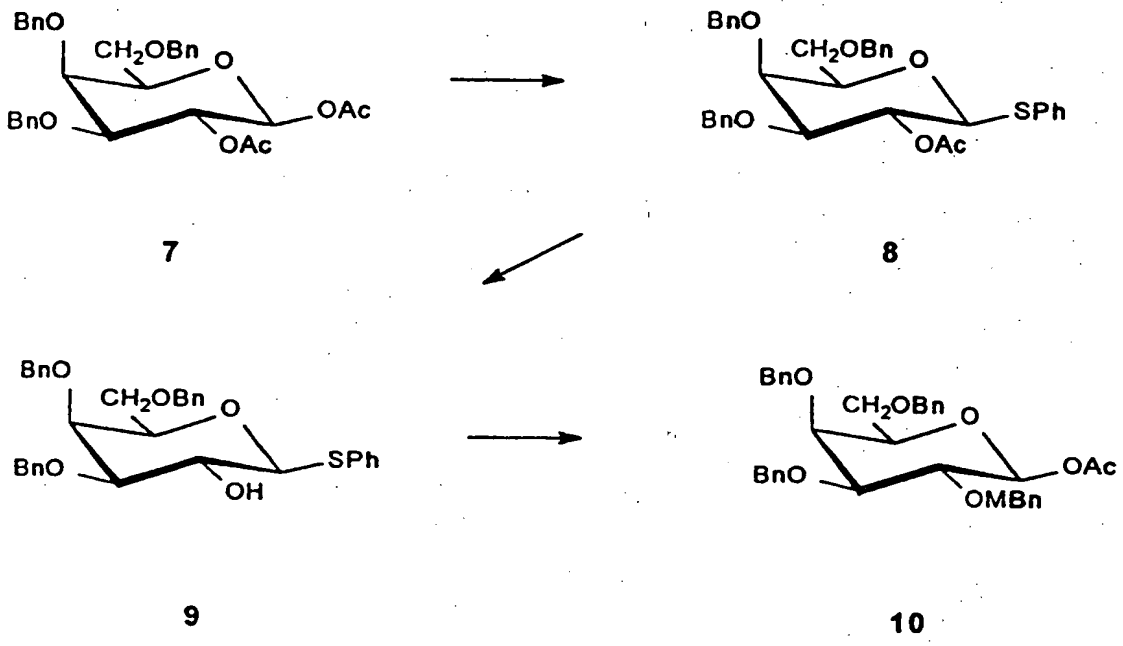


Fig. 4

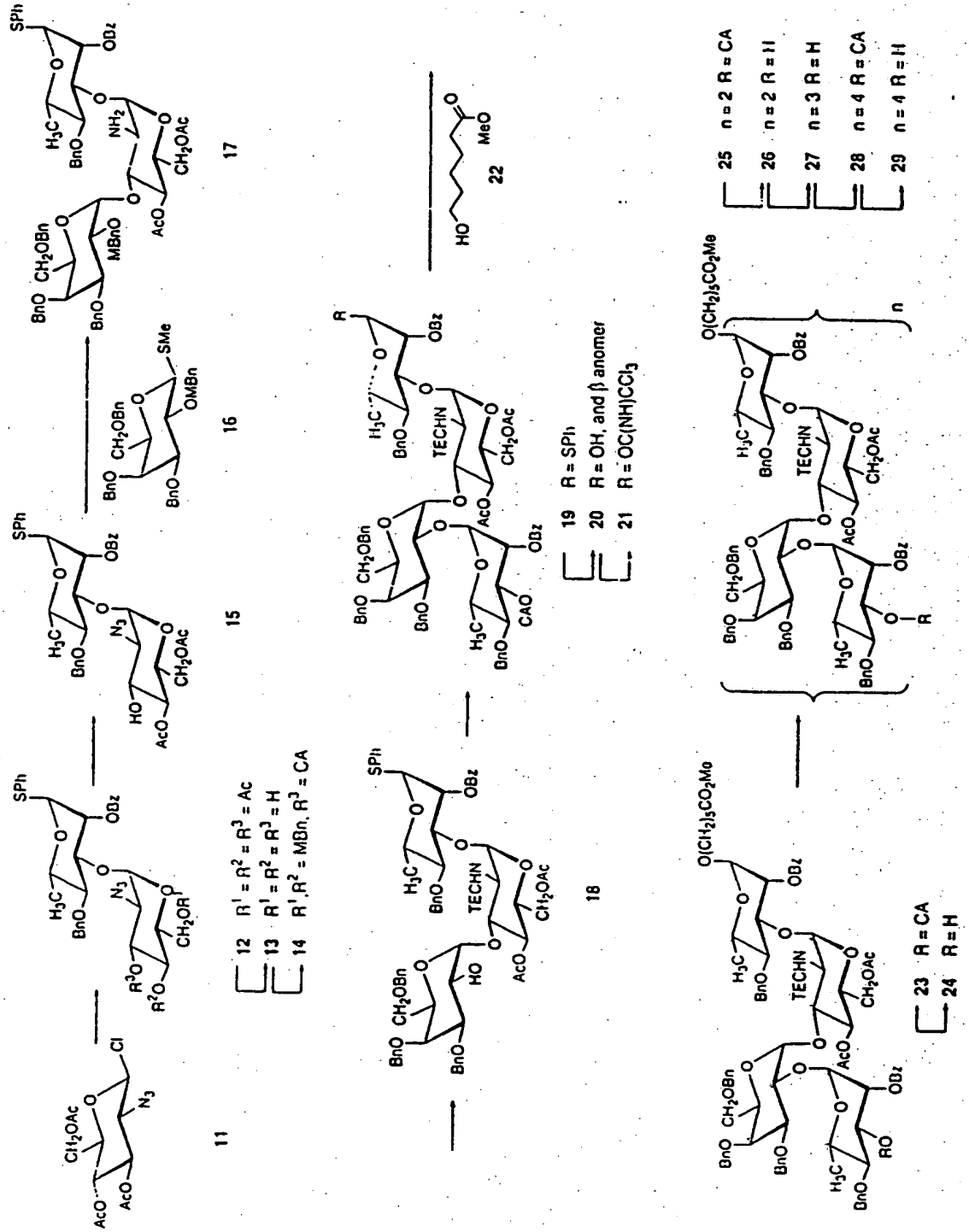


Fig. 5

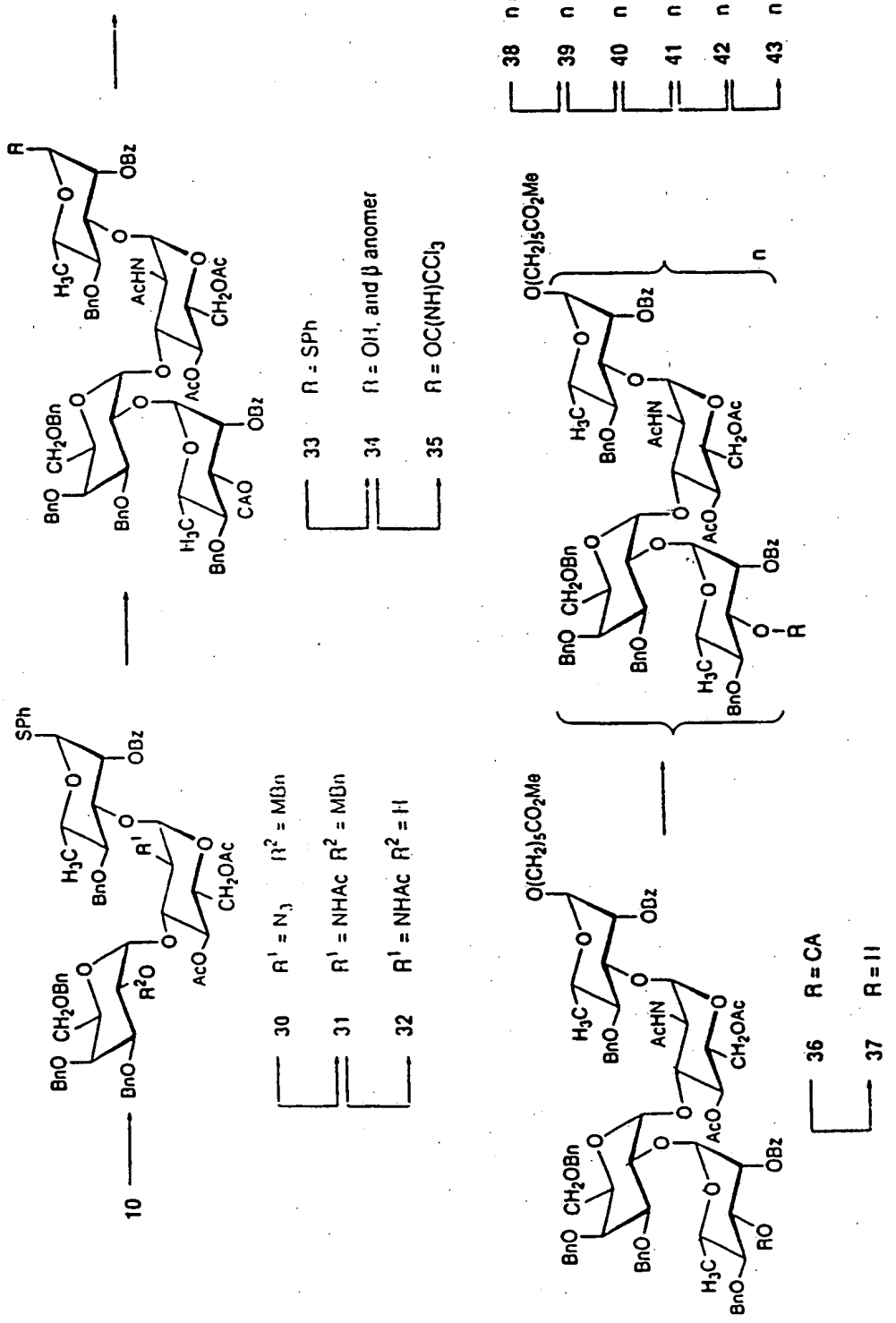
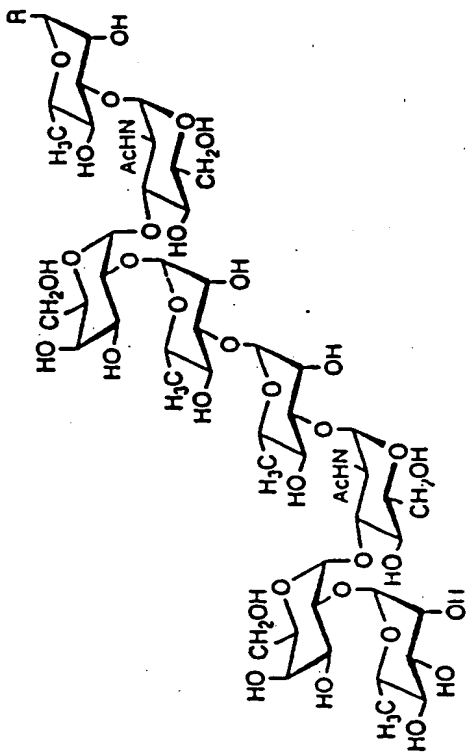
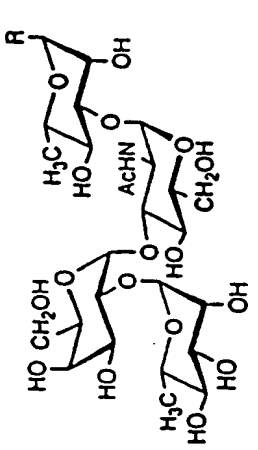


Fig. 6



46 R = O-(CH2)5-CO2Me

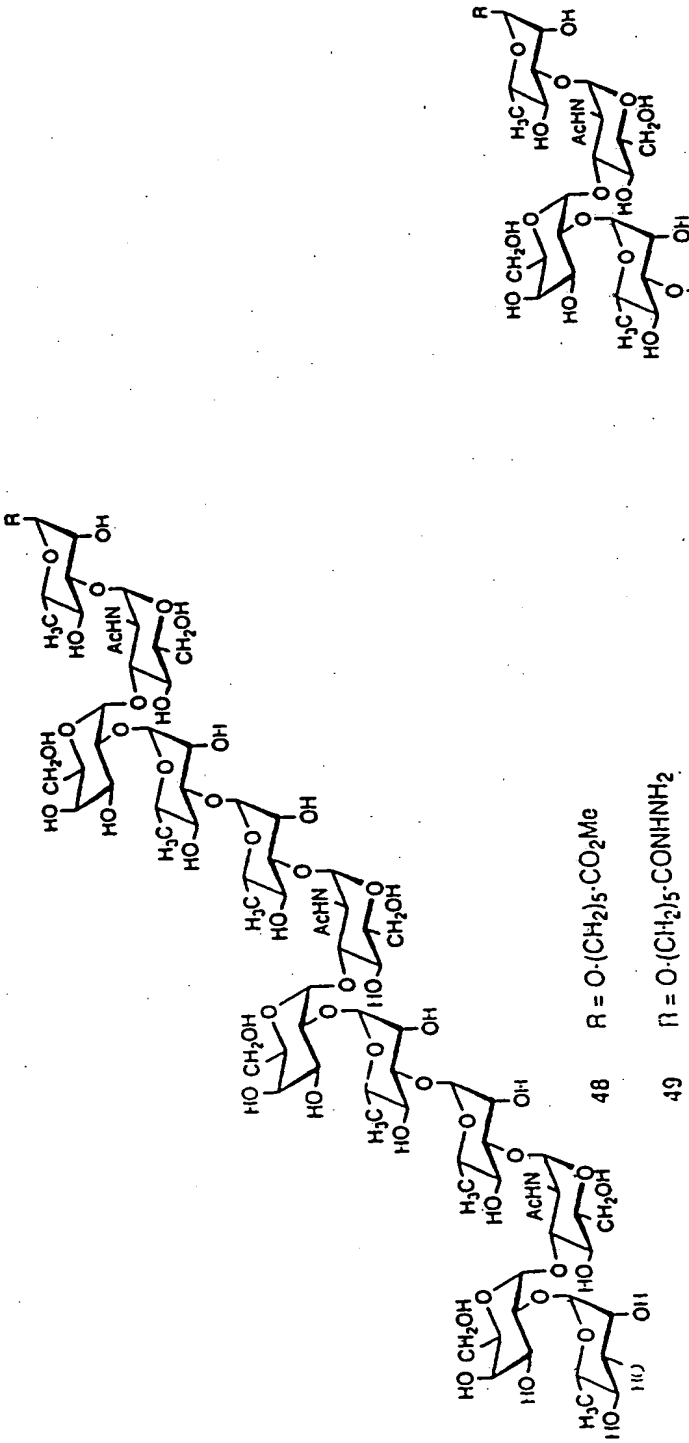
47 R = O-(CH2)5-CONHNH2



44 R = O-(CH2)5-CO2Me

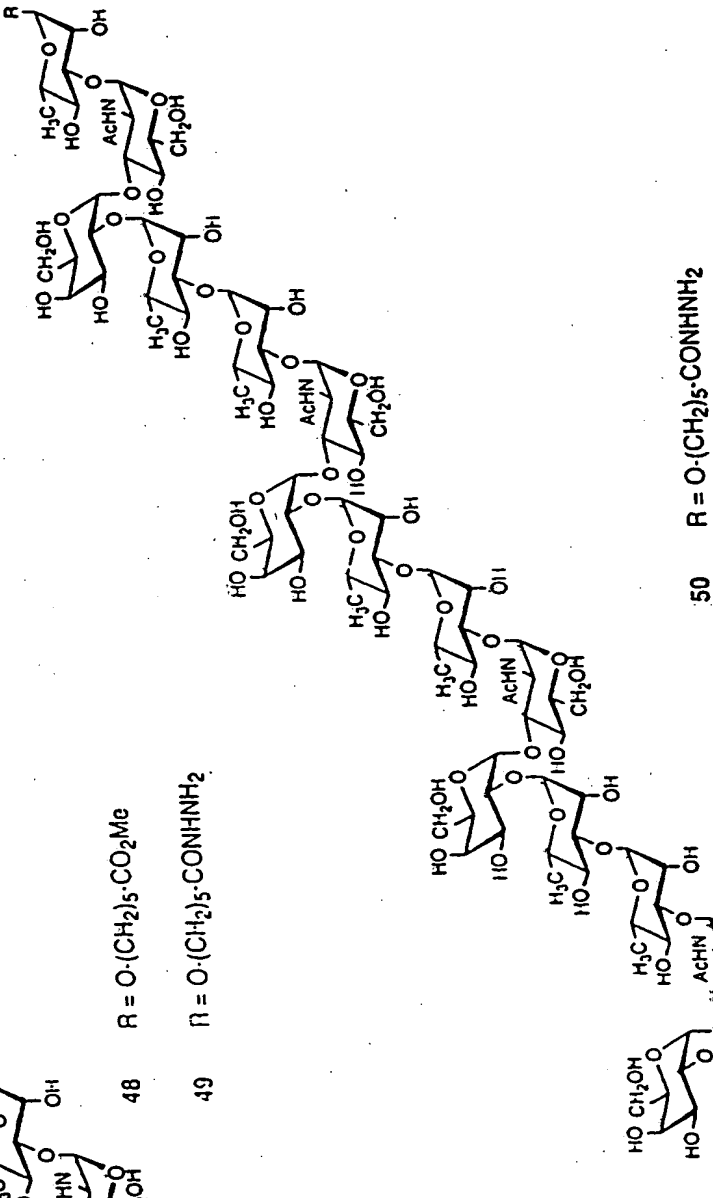
45 R = O-(CH2)5-CONHNH2

Fig. 7a



48 R = O-(CH2)5-CO2Me

49 R = O-(CH2)5-CONHNH2



50 R = O-(CH2)5-CONHNH2

Fig. 7b

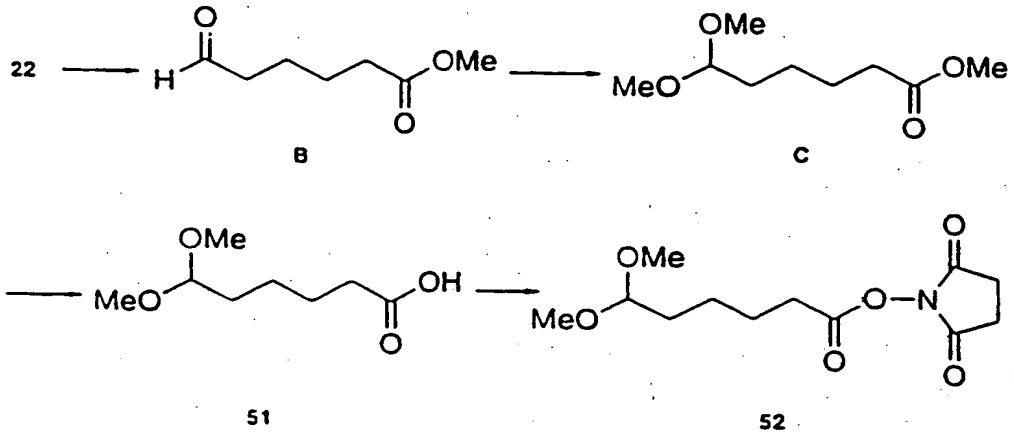


Fig. 8

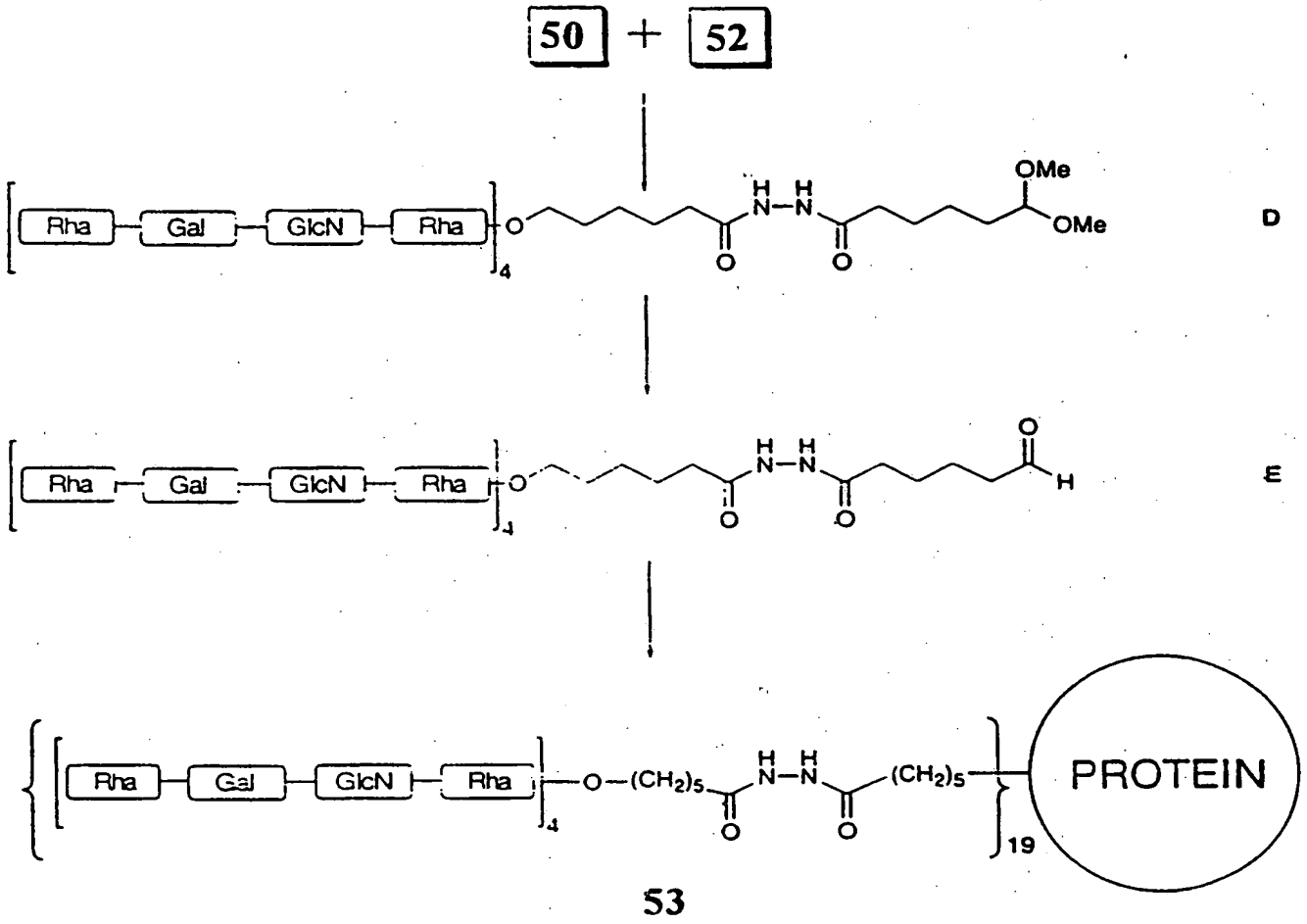


Fig. 9

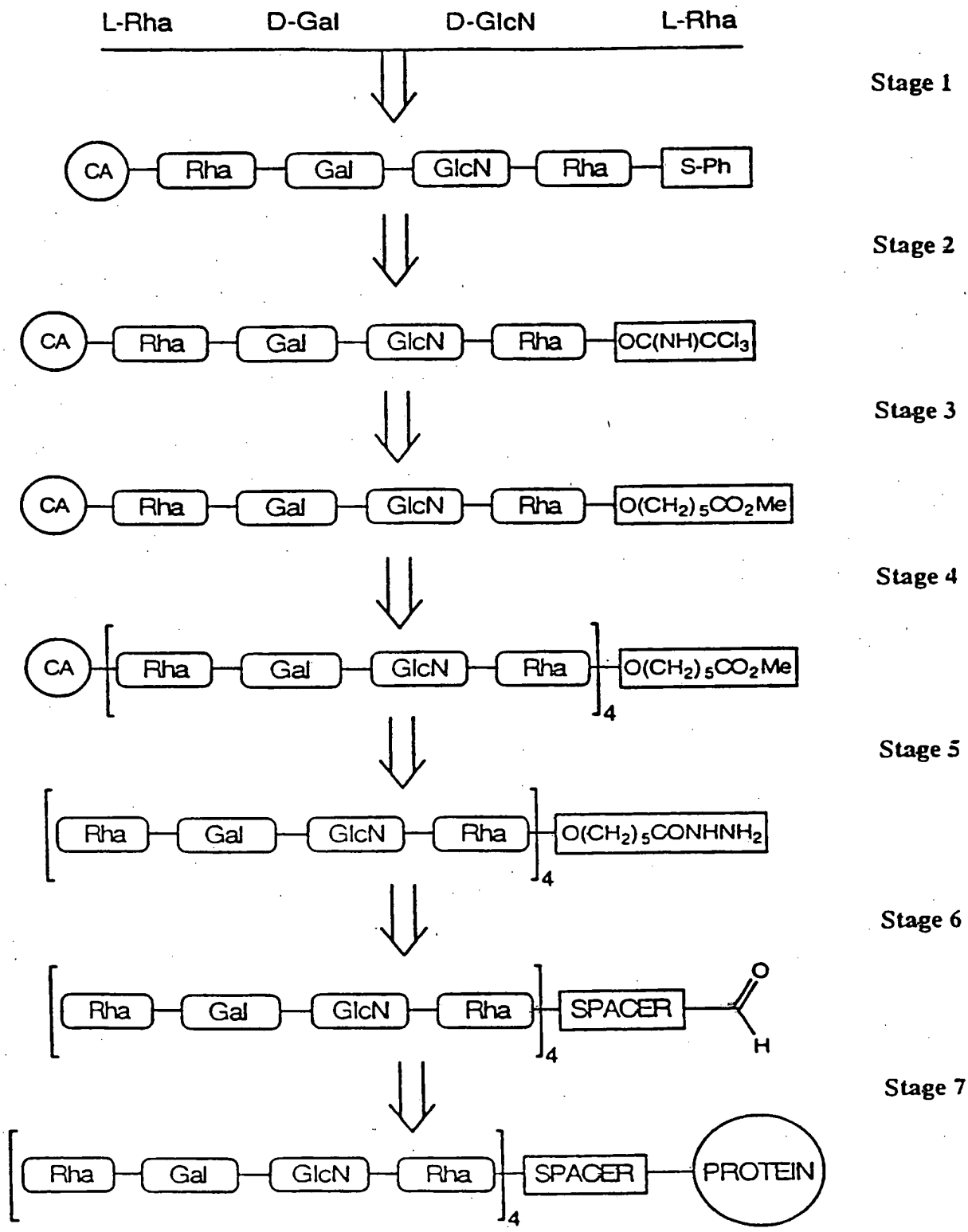


Fig. 10

INTERNATIONAL SEARCH REPORT

Interr ial Application No
PCT/US 98/14698

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H15/04 C07K16/12 A61K39/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H C07K A61K

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,X	CHU C. ET AL.: "Preparation, characterization, and immunogenity of conjugates composed of the O-specific polysaccharide of shigella dysenteriae type 1 (shiga's bacillus) bound to tetanus toxoid" INFECT. IMMUN., vol. 59, 1991, pages 4450-8, XP002079501 cited in the application see the whole document --- -/--	1-38

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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5 October 1998	20/10/1998

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	V. POZSGAY: "Synthesis of a hexadecasaccharide fragment of the O-polysaccharide of shigella dysenteriae type 1" J. AM. CHEM. SOC., vol. 117, 1995, pages 6673-81, XP002079502 cited in the application see the whole document	1-38
A	EP 0 254 905 A (YEDA RES & DEV) 3 February 1988	
A	QADRI F. ET AL: "Monoclonal antibodies specific for shigella dysenteriae serotype 13" DIAGN. MICROBIOL. INFECT. DIS., vol. 18, no. 3, 1994, pages 145-9, XP002079503	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr ial Application No
PCT/US 98/14698

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0254905 A	03-02-1988	CA 1291035 A	22-10-1991
		US 5204097 A	20-04-1993